Hospital Library

OCT 27 1936

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	Page
THE CURARIFORM ACTION OF ONIUM SALTS. *H. Raymond Ing*	527
THE CEREBRAL CIRCULATION. *H. G. Wolff*	545
CARDIAC METABOLISM. *E. W. H. Cruickshank*	597
THE BLOOD CALCIUM AND THE CALCIUM FACTOR IN BLOOD COAGULATION. *John H. Ferguson*	640
EFFECTS PRODUCED BY THE IRRADIATION OF PROTEINS AND AMINO ACIDS. *L. Earle Arnow*	671

VOL. 16, No. 4

Issued, October, 1936

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 8, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is owned and controlled by the American Physiological Society. It is edited by a board appointed by the society consisting of:

A. J. CARLSON, Chicago
GEORGE W. CORNER, Rochester
C. LOVATT EVANS, London
H. S. GASSER, New York
C. D. LEAKE, San Francisco
H. B. LEWIS, Ann Arbor
LEO LOEB, St. Louis

D. R. HOOKER, Managing Editor, Baltimore

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

Subscriptions should be sent to

D. R. HOOKER, *Managing Editor*
19 W. CHASE ST., BALTIMORE, MD.

NUTRITION ABSTRACTS & REVIEWS

Issued Under the Direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library

EDITORS

JOHN BOYD ORR, Imperial Bureau of Animal Nutrition, Aberdeen.
J. J. R. MACLEOD, University of Aberdeen.
HARRIETTE CHICK, Lister Institute, London.

The Editors, in co-operation with Corresponding Editors abroad scrutinize upwards of 400 periodicals and, with the assistance of a competent panel of abstractors issue abstracts of papers published throughout the World dealing with the subject of nutrition in all its aspects.

The abstracts are conveniently arranged in three main sections: (1) Chemistry and Physiology of Nutrition, (2) Human Diet in Relation to Health and Disease, and (3) Feeding of Domestic Animals. These sections are designed to meet the requirements respectively of the research worker, the medical practitioner and dietitian and the practical farmer.

Four numbers appearing in July, October, January and April of each year constitute a Volume, the subscription price for which is 21/–.

The current volume is Volume IV.

Subscriptions may be paid through any bookseller or direct to the Secretary, Committee of Management "Nutrition Abstracts & Reviews", Reid Library, Rowett Institute, Aberdeen, Scotland.

PHYSIOLOGICAL REVIEWS

VOL. 16 OCTOBER, 1936 No. 4

THE CURARIFORM ACTION OF ONIUM SALTS

H. RAYMOND ING

University College, London

The word onium is a convenient generic term for cations of the ammonium, phosphonium, sulphonium type in which the central atom satisfies its maximum covalency (for N, P, As, and Sb, 4; for S, 3; for I, 2, etc.) with carbon radicals and exerts as well one electrovalency. Simple examples are $(CH_3)_4N^{\bullet}$, $(CH_3)_4P^{\bullet}$, $(CH_3)_3S^{\bullet}$.

Onium salts have various pharmacological properties of which three are well-defined: 1, a curare-like paralysis of motor nerve endings in voluntary muscle; 2, stimulation of parasympathetic nerve endings (muscarine action), and 3, nicotine properties, *e.g.*, stimulating and depressant actions on sympathetic ganglia and contracture of striated muscle of the frog and fowl and of denervated mammalian muscle. In this review only the curariform properties will be considered.

No attempt will be made to review the extensive literature on curare, although some account will be given of curarine which is a quaternary base and appears to be the chief pharmacologically active alkaloid in curare.

The curariform properties of onium salts were discovered by Crum Brown and Fraser (1868/9). Their work has considerable historical importance since it constitutes one of the earliest attempts to make a systematic study of the relations between the chemical structure and pharmacological action of drugs. Their papers, written in the leisurely prose of the period, merit study and some account of their results will form the best introduction to our subject.

The object of their work was to discover the effect of making parallel chemical changes in a number of alkaloids, all of which had pronounced effects on the central nervous system. The chemical change chosen was the conversion of tertiary bases into quaternary metho salts by simple addition of methyl iodide: $R'R''R'''N + MeI \rightarrow R'R''R'''NMe\}$ I. The alkaloids investigated were strychnine, brucine, thebaine, codeine, morphine, atropine, nicotine and *N*-methylconiine. The methiodides and methosulphates of these alkaloids were all found 1, to be much

less toxic than their parent bases;[1] 2, to have lost entirely or to a large extent the central actions typical of their parent bases, and 3, with the possible exception of nicotine, to have a new property in common, viz., a curare-like paralysis of motor nerve endings in voluntary muscle.

The experiments were mainly performed on rabbits, but similar results were obtained with dogs, cats and frogs. The curariform nature of the action of these metho salts was clearly established by experiments similar to those of Cl. Bernard on curare. Thus, if the vessels to one leg of a frog were ligatured, the injection of strychnine methiodide produced complete paralysis of the whole animal except the ligatured leg which still responded to reflex stimulation; on the other hand, the paralysed leg still responded to direct stimulation of the muscle. Like curare these metho salts were inactive when given by mouth, and their effects could only be observed by injection. Moreover, the paralysis was reversible since animals recovered completely from paralysing but sub-lethal doses. Frogs survived large doses of these metho salts but mammals succumbed to large doses owing to paralysis of the respiratory muscles.

Crum Brown and Fraser also discovered that curariform activity was not confined to the metho salts of complex alkaloids. It was shown by etho salts and appeared to be a general property of "onium" salts since it was shown by the simplest quaternary ammonium salt, viz., tetramethylammonium chloride, and even by onium salts other than ammonium, *e.g.*, trimethylsulphonium chloride (1872).

Subsequent work, which will not be treated historically here, has served to establish even more firmly the accuracy of these original observations of Crum Brown and Fraser and to extend their generalisation to a great variety of onium salts. Curariform properties were demonstrated for phosphonium salts by Vulpian (1868) and Lindemann (1898), for arsonium and stibonium salts by Rabateau (1882) and for iodonium salts by Gottlieb (1894); cf. Ing and Wright (1933). The isolation of curarine from calabash curare by Boehm (1897) enabled him to confirm the curariform nature of onium salt action by direct comparison with the active alkaloid of curare (1910). His chemical investigations also made it clear that curarine was itself a quaternary base.[2]

Action on muscle and nerve. Cl. Bernard, Kölliker and other early workers noticed that large doses of curare sometimes depressed the

[1] That strychnine is converted into a much less pharmacologically active substance by methylation was first observed by Stahlschmidt (1859).

[2] For recent work on curare alkaloids see an important paper by King (1935).

direct excitability of striated muscle and of the nerve trunk. These effects were probably due to other substances, *e.g.*, potassium salts, in crude curare since Boehm (1910) clearly demonstrated that pure curarine chloride (1:1000) did not produce any progressive decline in the direct excitability of frog's striated muscle and Garten (1912) proved that curarine chloride was without effect on frog's sciatic nerve.

Simple onium salts, however, at relatively high concentrations may depress the excitability of striated muscle to direct stimulation (Höber and Waldenberg, 1909). This effect is similar to the depression of muscle produced by alkali metal salts, but paralysis to indirect stimulation is usually observed at concentrations well below that required to depress the direct excitability of muscle. A few onium salts produce contracture in striated muscles of the frog and the fowl. This is a "nicotine action"; it is quite independent of the curariform action and appears to be confined to onium cations which contain three methyl groups attached to the central atom (Ing and Wright, 1933).

Onium salts, with a few exceptions to be mentioned later, appear to be without effect on nerve trunks. Not many have been investigated in detail, but the cumulative weight of evidence for the strictly curariform nature of onium salt action is considerable. It always remained possible, however, that onium salt action was due to paralysis of the non-medullated terminations of motor fibres. Since medullated nerve appears to be more sensitive to the action of some drugs after asphyxiation (Graham and Gasser, 1931; Fromherz, 1933) Cowan and Ing (1933, 1935) investigated the action of onium salts on asphyxiated frog nerve; they found that tetramethylammonium, octyltrimethylammonium and methylstrychninium salts in 1–2 millimolar solutions had no appreciable effect on the action current even when the nerve was asphyxiated after treatment with these salts and then allowed to recover in oxygen. Curarine chlorine was similarly without effect. In the presence of silver chloride, however, onium salts may depress or even abolish the action current and this effect is hastened by asphyxia.

Action on invertebrates. There is some doubt whether curare and onium salts have an action on invertebrates similar to their action on vertebrates. Straub (1910) reported that curarine chloride was without action on earthworms. He also found that curarine had no specific action on neuromuscular junctions in many other invertebrates (quoted by Boehm, 1920, p. 183). Straub attributed the positive results of a few previous workers to their use of crude curare, which contains potassium salts. Cowan and Ing (1935) found that tetramethylammo-

nium and curarine chlorides did not reduce the response of crab nerve-muscle preparations (*Maia squinado*) to indirect stimulation even at concentrations (10 and 3 millimolar, respectively) far exceeding those needed to paralyse frog nerve-muscle preparations. Katz (1935) also found curare without effect on the indirect excitability of the limb muscles of *Carcinus mænas*, although magnesium salts easily produced complete "curarization." Gaskell (1914), however, reported prolonged paralysis of the leech by curare. The dose required for complete paralysis was large (2 mgm.) and about a month was required for complete recovery. He was able to show that the paralysis was of the type normal in vertebrates; voluntary muscles were paralysed to indirect stimulation, but contracted normally to direct stimulation; involuntary muscles were unaffected. Gaskell attributed Straub's results on the earthworm to his using too small a dose; Straub's maximum dose was 0.1 mgm. pure curarine chloride, which corresponds to about 1–2.5 mgm. of a good specimen of curare. Curare also antagonises the contracture of leech muscle produced by acetylcholine.

Cowan and Ing (1935) found curarine, strychnine metho- and ethochlorides and tetramethylammonium chloride without any appreciable effect on the action current in *Maia* nerve, unless silver salts were present. Sea water saturated with silver chloride depressed the action current in crab nerve slightly, but the addition to it of tetramethylammonium or curarine chlorides abolished the action current rapidly.

Non-curariform onium salts. It would be easier to enumerate onium salts which do not have any curariform activity than those which possess it in some degree; almost all types of quaternary ammonium salts and simple phosphonium, arsonium, stibonium, sulphonium and iodonium salts have been found to be curariform. The reader is referred to an article by Trendelenburg (1923) for a detailed account of individual onium cations. Exceptions are provided by betaines which are pharmacologically inert. The metho salts of a few alkaloids also appear to have very slight curariform activity or to be devoid of it. Crum Brown and Fraser concluded that nicotine metho salts had no true paralysing action on motor nerve endings. Pohl (1904) found that the methochlorides of papaverine and papaveraldine had no curariform activity on frogs. Pak and Read (1935) have stated recently that *N*-methylephedrine metho salts do not produce curariform paralysis in cats and dogs. A more interesting exception is provided by prostigmin, $m\text{-}Me_2N\cdot CO_2\cdot C_6H_4\cdot NMe_3\}SO_4Me$, which like eserine antagonises curare (Aeschlimann and Reinert, 1931). Since phenyltrimethylammo-

nium salts are strongly curariform (Ing and Wright, 1931), the introduction of the dimethylurethane group in prostigmin has produced a striking change in the pharmacological properties. Prostigmin may prove to be a member of a large class of similar onium salts, since Stedman (1926, 1929) has prepared numerous synthetic urethanes resembling eserine in their pharmacological properties and quaternary salts derived from them often retain the characteristic eserine-like properties.

With these few exceptions, curariform activity must be regarded as a general property of onium salts and consequently it is of interest to study the relative activities of different classes of onium salts.

Measurement of curariform activity. It is always difficult to study quantitatively a pharmacological reaction which involves the cessation of some physiological response. The usual method consists in measuring the activity of the tissue at suitable time intervals until paralysis is complete for different concentrations of the drug. In this way both action-time and concentration-time curves may be plotted.

Boehm (1910) and Trendelenburg (1923) estimated the limiting concentrations of many onium salts which just failed to produce complete paralysis of isolated frog nerve-muscle preparations. Kulz (1922) estimated the minimum concentrations of alkyltrimethyl- and alkyltriethyl-ammonium salts required to paralyse similar preparations. The disadvantage of these methods is that the time factor is neglected. Paralysis does not occur suddenly, but occupies a time interval during which the muscle executes weakened contractions to indirect stimulation. The times required for complete paralysis with high dilutions of onium salts are considerable and during this period isolated preparations may deteriorate. In practice such times also vary greatly among themselves as Boehm's records show. Moreover, Ing and Wright (1931) found that frog nerve-sartorius preparations recovered slowly when immersed in tetramethylammonium solutions too dilute to achieve complete paralysis. For these reasons observations over extended time intervals are rather unreliable.

Many authors have compared the action of onium salts on intact or decerebrate animals; *e.g.*, Marshall (1914/16) studied the effect of successive replacement of methyl by ethyl groups in tetramethylammonium by measuring the doses required to paralyse (prevent turning over) decerebrate frogs within a given time. The value of results obtained by such methods is obscured by uncertainty about the rates of excretion of different ions. Cowan and Ing (1934) found that curarine chloride and strychnine methochloride were equally active on the

isolated frog's sartorius preparation, but in the decerebrate animal strychnine methochloride was much less active because it was much more rapidly excreted by the kidneys.

Peiser (1920) estimated the activities of a few tetraalkylammonium salts by measuring the times required for complete paralysis of isolated frog's sciatic-gastrocnemii with equimolar solutions. This method takes no account of possible differences in the rates of diffusion of different ions into the muscle.

Boehm (1910) discovered two interesting facts about the diffusion of onium salts into muscle which have importance in this connection. He found that the time of poisoning of an isolated frog's gastrocnemius was to some extent independent of the time of immersion of the preparation in the drug solution. Times of immersion of 1 to 30 seconds in 0.1 per cent curarine chloride resulted in approximately the same time for complete paralysis (60–80 min.). Increasing the immersion time to 1 minute lowered the mean time for paralysis to 43 minutes, whilst immersion times of 3 to 17 minutes only lowered the mean time for complete paralysis to 27 minutes. Ing and Wright (1931) found similar effects for tetramethylammonium in the isolated frog's nerve-sartorius preparation. It is clear that the drug action involves a very rapid diffusion of the salt into the muscle, which is followed by the slower paralysis. Moreover, the drug is in some way bound by the tissues since it cannot diffuse out sufficiently rapidly to prevent the onset of paralysis. The slower paralysis process probably represents a second diffusion process within the tissues, viz., the attainment of effective concentrations of the drug at the sites of action.

Boehm also found that many onium salts which were actually weaker than curarine paralysed an isolated frog's gastrocnemius more rapidly than the latter at some concentrations; thus, 0.1 per cent tetramethylammonium paralysed a preparation in a third the time of 0.1 per cent curarine and for 0.01 per cent solutions the time ratio was two-thirds; but tetramethylammonium failed to achieve complete paralysis at 0.005 per cent, whereas curarine began to fail at 0.000,01 per cent. The ratios quoted are exaggerated by Boehm's use of percentage instead of molar concentrations, but it is reasonable to suppose that small ions will diffuse in the muscle more rapidly than relatively large ones and consequently may appear more active at certain dilutions.

Ing and Wright (1931) tried to avoid the difficulties arising from different diffusion rates by using the frog's sartorius, since this muscle is only a millimetre or so thick even in large frogs. They were able to

show that the paralysis times for perfused and isolated sartorii were identical within the limits of experimental error and consequently the diffusion factor is negligible for isolated sartorii. The paralysis time for an isolated gastrocnemius was, however, much longer than that for the perfused muscle; consequently, with gastrocnemii the diffusion factor is not negligible and comparative experiments on this muscle are liable to error arising from the different rates of diffusion of large and small ions.

The isolated nerve-sartorius preparation (*R. esculenta*) gives astonishingly reproducible results. The times of poisoning are short and the preparation recovers completely when washed with Ringer's solution; consequently, one preparation can be used to test a variety of compounds. If the height of the contraction recorded by an isometric lever be plotted against time, a sigmoid action-time curve is obtained which is almost linear over its middle range. High dilutions of onium salts show a well-defined latent period at the beginning when apparently no action has occurred and the curve may later flatten out if incomplete paralysis is achieved. In comparing the paralysis times for equimolar concentrations it is important to choose concentrations which do not give very short or very long times. This is illustrated by some of Ing and Wright's data (1933): *e.g.*, for short paralysis times (3–4 min.) strychnine methiodide appears to be only twice as active as tetramethylammonium iodide, but for longer times (6–25 min.) it is ten times as active; for still longer times tetramethylammonium may fail to achieve complete paralysis at concentrations at which strychnine methiodide is still effective. The reason for the choice of intermediate times is obvious; for short times the minimum time within which action can be produced may become significant and for long times the dose may approach the minimum concentration which just fails to produce any action. Clark (1933, p. 85) has in fact shown that concentration-time curves derived from Ing and Wright's data will fit equally well two types of formula, viz., $c(t - t_o) =$ constant and $(c - c_o)^n t =$ constant, where $c =$ concentration, $t =$ time and n, c_o and t_o are empirical constants.

Ing and Wright compared the activities of many onium salts by measuring the times of paralysis for equimolar concentrations (1931, 1933). Laidlaw (1913) compared the activities of stereoisomeric canadine methochlorides by measuring the concentrations which gave identical action-time curves. He used the perfused hind limbs of frogs and recorded the contraction of the gastrocnemius to indirect stimulation. His method appears to be the most satisfactory so far devised, but it is

less well adapted to the comparison of a large number of compounds since he found it impossible to make more than one estimation on each hind limb. The chief disadvantage of Ing and Wright's method is that the standard of comparison is a time interval. The paralysing action is almost certainly a complex chain of physicochemical events and the time for complete paralysis has no clear significance. Laidlaw's method eliminates possible variations in the times of a number of processes by a trial and error estimation of concentrations which reproduce identical action-time curves. The chief advantages of Ing and Wright's method are its speed, its reproducibility and its use of one preparation for several determinations.

Chemical structure and curariform activity. In considering the relations between curariform activity and detailed chemical structure among onium cations, it is difficult to make comparisons over a large field because workers have used different methods of estimating activity. The most striking feature, however, is the extent to which curariform activity is independent of detailed chemical structure. Of some forty different cations examined by Ing and Wright seventeen had approximately equal activity. These ions differed both in the nature of the carbon radicals and in that of the central atom, as will be clear from the following list:

Tetra-alkylonium ions: tetramethyl- and tetrabutyl-ammonium; tetrapropyl- and tetrabutyl-phosphonium; tetraethyl- and tetrapropyl-arsonium; *n*-butyl-, *n*-amyl-, *n*-hexyl-, *n*-heptyl- and *n*-octyl-trimethyl-ammonium.

Arylonium ions: phenyltrimethyl- and phenylbenzyldimethyl-ammonium; diphenyliodonium; *n*-propyl-, *n*-butyl- and *n*-octyl-quinolinium. One millimolar solutions of all these ions paralysed a nerve-sartorius preparation (*R. esculenta*) in 6 $\pm$0.5 minutes at room temperature (15°–18°C.)

There is, however, a small number of much less active ions, *e.g.*, methyltriethyl- and tetraethyl-ammonium, triethylsulphonium, tetramethylarsonium and methyl-pyridinium and -quinolinium. Of these, the first three had no action on a nerve-sartorius preparation at 1 millimol per litre and the last three did not produce complete paralysis at this concentration.

Tetraethylammonium has long been known to be anomalous in its curariform properties. Its activity has been variously estimated as from 1/100th (Santesson and Koraen, 1900) to 1/25th (Boehm, 1910) of that of tetramethylammonium. Marshall (1914/16) made a careful study of its properties. He found that the successive replacement of

methyl by ethyl groups in tetramethylammonium led to a rapid decline in activity, trimethylethyl- having about 1/3rd, dimethyldiethyl- about 1/25th and methyltriethyl- and tetraethyl-ammonium both about 1/80th of the activity of tetramethylammonium. His results were confirmed by Ing and Wright (1931) who found, if anything, a wider divergence in activity among members of this series.

Tetraethylammonium also differs qualitatively in its pharmacodynamic properties. It produces fibrillary twitching in voluntary muscle and large doses produce irregular contractions. These effects were noticed by several early workers, but were first studied in detail by Marshall (1914/16) who gives early references. Marshall regarded the muscle tremors as due to an action on the myoneural junction. He found that they were antagonised by curare, tetramethylammonium and calcium. Rothberger (1902) had previously recorded that tetraethylammonium would antagonise curare paralysis in cats, but four out of his eight experiments were negative. It is more probable, however, that the muscle tremors and contractions are due to a direct action upon the motor nerve fibres. Marshall himself showed that after the injection of tetraethylammonium into pithed frogs, the sciatic nerve responded to stimuli smaller than the minimal stimulus necessary before the injection. Loeb and Ewald (1916) found that muscle tremors and twitching were produced by soaking only the nerve of frog nerve-muscle preparations (*R. pipens*) in M/160–M/24 tetraethylammonium salt solutions, and this effect was antagonised by calcium. Ing and Wright (1931) noted that tetraethylammonium produced an initial increase in the response of nerve-muscle preparations to indirect stimulation and Cowan and Ing (1933) found that this ion increased the action current (as measured by area under the galvanometer deflection × time curve) in frog sciatic nerve by 50–100 per cent. Cowan and Walter (1935) using a Matthews oscillograph have shown that tetraethylammonium (10 millimolar) prolongs the negative after-potential and gives rise to repetitive discharges in frog sciatic nerve in response to single condenser discharge shocks; higher concentrations also induce spontaneous asynchronous activity in the nerve. They attribute these effects to reduction in the 'accommodation' of the nerve, which renders it very sensitive to stimuli of long duration.

It is a matter of definition whether tetraethylammonium should be regarded as truly curariform. Marshall found that paralysis to indirect stimulation could be achieved by suitable concentrations in frogs without sensible reduction in the direct excitability of muscle. Its action is perhaps best regarded as a dual one, a weakly curariform action

on the neuromuscular junction and a direct action on the motor nerve fibres.

The other weakly active ions mentioned above have not been so closely investigated. Marshall drew attention to the similarity between methyltriethyl- and tetraethylammonium. Ing and Wright found triethylsulphonium slightly weaker than tetraethylammonium in curariform activity; it also produced muscle tremors and increased the initial response of isolated preparations to indirect stimulation.

The anomalous properties of tetraethylammonium and similar ions are not due to any peculiar property of ethyl groups. Replacement of methyl by ethyl groups in onium ions frequently leads to a diminution of curariform activity, *e.g.*, in tetra-alkyl-ammonium ions and in all alkaloid metho ions so far investigated, but this is not invariably true. Methyl-pyridinium and -quinolinium are not only much weaker in curariform activity than the corresponding ethyl and higher alkyl ions but the former are also the only alkyl-pyridinium and -quinolinium ions which produce muscle tremors and an initially increased response in isolated preparations. In the arsonium series the tetramethyl member is nearly as weak in curariform activity as tetraethylammonium, whereas the tetraethyl member is quantitatively similar to tetramethylammonium.

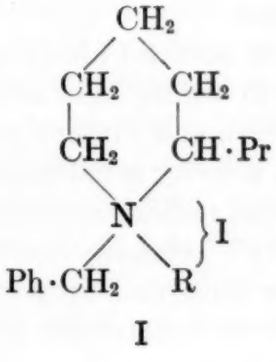
The production of muscle tremors by onium ions has only been observed with the very feebly curariform ions, but there may be no necessary connection between the two properties. Marshall noted that tetraethylammonium produced muscle tremors more readily than the equally curariform methyltriethylammonium and Ing and Wright did not observe muscle tremors in preparations poisoned with tetramethylarsonium, nor any initial increase in the response to indirect stimulation (unpublished data).

None of the ions so far discussed is appreciably more active than the simplest, viz., tetramethylammonium, and all of them are much less active than curarine. It is difficult to compare curarine quantitatively with simple onium ions; at 0.1 millimol per litre it is some ten times as active as tetramethylammonium, but at greater dilutions its activity remains high whereas simple onium ions fail to achieve complete paralysis. The limiting concentration of tetramethylammonium chloride which just fails to produce complete paralysis of isolated frog nerve-muscle preparations is 0.005 per cent, whereas the corresponding value for curarine chloride is 0.000,01 per cent (Boehm, 1910). The only onium salts which approach curarine in activity appear to be the metho salts of certain alkaloids. Cowan and Ing (1934) found strychnine

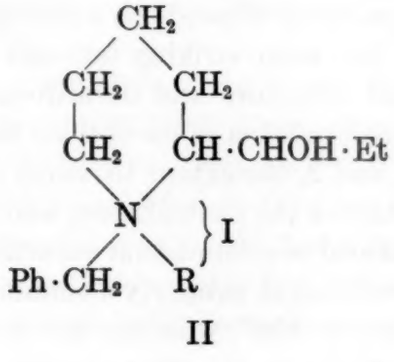
methiodide slightly more active on the frog's sartorius preparation than curarine chloride. On decerebrate frogs, however, it was considerably less active because much more rapidly excreted by the kidneys than curarine. Strychnine ethiodide has about half the activity of the methiodide on isolated preparations (Ing and Wright, 1933). Cowan and Ing used Boehm's formula, $C_{19}H_{25}ON_2Cl$, for curarine chloride, but it is probable that the molecular weight is of the order of twice that for this formula, since the formula of tubocurarine is $C_{38}H_{44}O_6N_2Cl_2$ (King, 1935); this would mean that curarine chloride was about twice as active as strychnine methochloride at 0.1 to 1.0 millimol per litre.

Among other types of onium salts, it may be noted that choline chloride is very weakly active, 0.35 per cent solutions failing to achieve complete paralysis of isolated frog nerve-muscle preparations (Boehm, 1910); acetylcholine is 6 to 10 times more active (Trendelenburg, 1923). Choline chloride also appears to antagonize curare paralysis (Pal, 1910; Abderhalden and Müller, 1910). Neurine has about the same activity as trimethylethylammonium, *i.e.*, about half that of tetramethylammonium. Only two amine oxides appear to have been examined, viz., trimethylamine and methylethylaniline oxides, both of which had very feeble curariform activity (Ing and Wright, 1931).

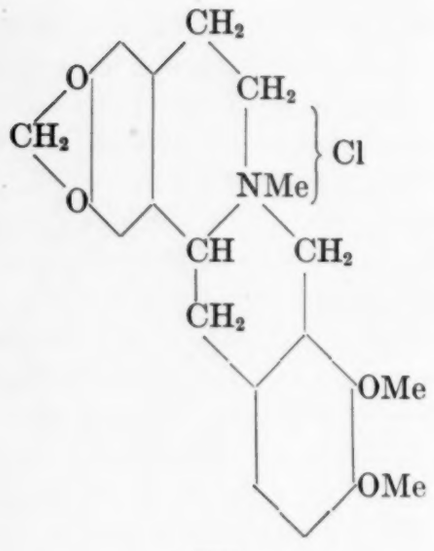
Not many stereoisomeric onium salts have been investigated. Hildebrandt (1905) examined a number of alkylbenzylconinium salts (I) which in virtue of asymmetric carbon and nitrogen atoms exist in two racemic forms. He estimated the minimum doses required to paralyse frogs and found that for each of the ethyl-, propyl-, butyl- and *iso*amyl-benzylconinium iodides the higher melting β-isomer was more active than the lower melting α-isomer. The differences were slight and in no example was the ratio $\beta:\alpha$ greater than 2:1. With ethyl-, propyl- and *iso*amyl-benzylconhydrinium iodides (II) the differences between the activities of α- and β-isomers were also slight but irregular. In these salts



I



II



III

(R = C_2H_5, C_3H_7, C_4H_9, or C_5H_{11})

there are three asymmetric atoms.

A better example is provided by Laidlaw's experiments (1913) on the stereoisomeric canadine methochlorides (III), which contain one asymmetric nitrogen and one asymmetric carbon atom and consequently exist in two racemic forms, α and β, and four optically active forms. Laidlaw compared the curariform activities of the pure α and β laevo isomers and of the pure α and β racemic isomers by the method already mentioned above. He found the following ratios: l–α:l–β = 1:12; l–α:dl–α = 1:5; and l–β:dl–β = 1:4; from these results it is easily calculated that the four optically active isomers have the following relative activities: l–α, 1; d–α, 9; l–β, 12; d–β, 28. Laidlaw's curves show that the weakest isomer, viz., l–α, is much more active than simple quaternary ammonium salts and is comparable with strychnine metho salts. There appear to be no data for optically active onium salts in which the asymmetry depends on a nitrogen atom only.

The two most striking features of the data outlined above relating chemical structure and curariform activity in onium salts are 1, the very small number of exceptions to Crum Brown and Fraser's generalisation, and 2, the extent to which curariform activity is independent of the nature of the central atom and of the carbon radicals attached to it. It is natural to assume that curariform activity is primarily due to some physicochemical property common to onium ions, and Meyer (1902) and Führer (1907) were the first to emphasize the dependence of curariform activity on the strong basicity of onium bases.

A mild degree of curariform activity is commonly associated with organic bases, *e.g.*, simple aliphatic amines (Brunton and Cash, 1884) and alkaloids such as coniine, sparteine, etc., which contain no specific pharmacodynamically active groups. The salts of such bases are hydrolysed in aqueous solution and the free bases readily penetrate cells. Onium salts, however, are not hydrolysed, the free bases being comparable with alkali hydroxides, and Overton (1897) and Harvey (1911) found that quaternary ammonium salts penetrate cells extremely slowly, if at all.

The contrast between bases which penetrate cells easily and those which do so with difficulty is well illustrated by an experiment of Ing and Wright (1931). A frog's nerve-sartorius preparation was treated with a 10 millimolar solution of ammonium chloride; full action (60 per cent inhibition of the response to indirect stimulation) occurred in 10 minutes, but when the preparation was left immersed in the drug solution complete recovery occurred within an hour. A similar preparation was then immersed in 0.07 millimolar tetramethylammonium iodide; full action (70 per cent inhibition) occurred in an hour, but the preparation had only recovered 75 per cent of its original activity in 4 hours.

Fränkel (1904) suggested that the curariform properties of onium salts were caused by the new spatial orientation which the utilisation of the two potential valencies of tertiary bases in onium salt formation involved. This cannot be true since the spatial orientation of quaternary ions is not different from that in ammonium. The significant fact appears to be the saturation of the maximum covalency of the central atom with carbon radicals which produces a cationic stability not found in the cations of amine salts. Whereas cations of the type, $R_3NH^{\oplus}$, are readily attacked by hydroxyl ions, thus:

$$R_3NH^{\oplus} + OH^{\ominus} \rightleftharpoons R_3N + H_2O,$$

onium cations are quite stable in aqueous solution and resemble alkali metal cations. The free bases rarely decompose below 100°C. and the salts are in general even more stable.

The fact originally observed by Crum Brown and Fraser that metho salts derived from alkaloids with highly specific pharmacodynamic properties are found to have lost entirely or to a large extent the properties of their parent bases is also readily explained on the lines suggested above. It is a commonplace of pharmacology that the pharmacodynamic properties of alkaloids are usually due to the free bases and not to the cations of their salts; this has frequently been demonstrated for

local anesthetics and, *e.g.*, cocaine metho salts have no local anesthetic properties (Ehrlich and Einhorn, 1894).

There are, however, some exceptions to Crum Brown and Fraser's generalisation; *e.g.*, strychnine metho salts appear to have some stimulating action on the central nervous system resembling that of the parent base, but the extent of this action depends on the mode of administration and it is naturally not observed when the peripheral paralysis is complete (Cowan and Ing, 1934; Crum Brown and Fraser, 1868/9; Jolyet and Cahours, 1868).

Atropine provides a more notable exception; Crum Brown and Fraser found that atropine metho salts retained the typical peripheral actions of the parent base, but showed no convulsant properties; the curariform properties were also well-marked. Atropine metho salts have in fact a stronger action on peripheral structures than the parent base. Issekutz (1917) reported that atropine metho salts acted about 8 times as powerfully as atropine on the frog's heart and 3 to 4 times as powerfully on the salivary glands. Cushny (1920) estimated that atropine methobromide was about 1.5 times as powerful as atropine in antagonizing the action of pilocarpine on the salivary glands of the dog. It may be noted that atropine etho salts have weaker peripheral activities than atropine.

Curariform activity therefore appears to depend primarily on the ionic character of onium cations and not on their detailed chemical structure. Such a view makes plausible the generality of the effect and accounts for the diversity of ions with quantitatively similar activities.

It remains to consider onium ions which show abnormally high or low activities. The structure of curarine is unknown (compare King (1935) for work on the structure of tubocurarine) and that of strychnine, canadine, etc., is too complex to make profitable any speculation on the structural factors which contribute to the high activity of their metho salts.

The abnormally low activities of tetraethylammonium and a few similar ions are very difficult to explain.[3] They are not due to any peculiar properties of ethyl groups. There is no evidence for any abnormal physical properties of tetraethylammonium and similar ions. Ing and Wright (1933) suggested that the anomalous curariform proper-

[3] Tetraethylammonium has frequently been regarded as anomalous with respect to the muscarine-like and nicotine-like properties of onium ions, but a survey of the available data shows that this is not true. It behaves similarly to a large number of onium salts which contain less than three methyl groups on nitrogen.

ties might be due to the unique value of some normal physical property, such as ionic volume. They found that ions of the type, $R_4X^{\oplus}$, showed minimum activity for certain sizes of R and X, and that for larger atoms X the minimum moves towards ions with smaller radicals R. Thus in the ammonium series $Me_4N = Bu_4N > Pr_4N > Et_4N$; in the phosphonium series $Bu_4P = Pr_4P > Me_4P > Et_4P$; and in the arsonium series $Pr_4As = Et_4As > Me_4As$. The weakest ions Et_4N, Me_4As, and Et_3S have radii between 2.3 and 2.8 Å. U. and ions with radii smaller or greater than these limits have greater curariform activity. Such considerations of size can only apply to ions of the same shape; *e.g.*, the isomeric ions, tetraethyl- and amyltrimethyl-ammonium must have approximately equal ionic volumes, but their contours will be different and their curariform activities are widely different. It is, however, difficult to give any physiological meaning to such considerations of size and shape. Pharmacologists are familiar with numerous examples of maximum activity in one member of a homologous series of compounds and it has frequently been suggested that such maxima may be due to an optimal "fit" between the drug and the tissue upon which it acts. The example presented by tetraethylammonium, however, is one of minimum activity for one member and greater and more or less uniform activity for its higher and lower homologues.

Theories of curariform activity. The nature of the ionic reaction involved in curariform activity is completely unknown. Laidlaw's discovery of the wide divergence in activity of the optical enantiomorphs of α- and β-canadine methochlorides suggests that a chemical reaction is involved. Ing and Wright (1931) measured the temperature coefficient of the action of tetramethylammonium iodide on frog nerve-muscle preparations for the temperature ranges, 23.5° to 13.5° and 13.5° to 3.5°C., and found $Q_{10} = 1.5$, a value consistent with the possibility of a chemical reaction being involved. The nature of the chemical reaction postulated remains a matter for speculation. On general chemical grounds the possibilities are rather limited. Any decomposition of onium salts at the site of action is made improbable by the extent to which curariform activity is independent of detailed chemical structure. There remains the possibility of some ionic exchange or permutit-like reaction in which the onium ion replaces some normal cation in the structure upon which the drug acts (Ing and Wright, 1931).

Cl. Bernard's original view of the action of curare was that the drug had a local action on that part of the motor nerve which lies within the muscle. When it was discovered that the terminations of motor nerves

in striated muscle had a characteristic histological appearance, it was assumed that the nerve endings might have properties different from those of the fibres which they terminated and might consequently react differently to drugs. Since curarine had no action on motor fibres, the nerve endings were assumed to be the site of its action. Later Langley (1906) introduced the idea of a "receptive substance" in the muscle which receives the stimulus from the nerves and by transmitting it causes contraction. Curare was then supposed to paralyse the action of the receptive substance. This idea was based on Langley's studies on the nicotine-curare antagonism and in particular on his observation that curare antagonised the nicotine contracture of frog muscle even after the degeneration of the motor fibres and of their terminations. Since the analogy between the action of curarine and that of onium salts appears to be complete, it has naturally been assumed that onium salts also act upon the receptive substance.[4]

The development of the neurohumoral or chemical transmission theory of nervous stimulation which originated in Loewi's classical experiments has modified profoundly current ideas of nerve endings and of the action of drugs at nerve endings. Evidence for the view that the transmission of nerve impulses from motor nerves to striated muscle involves the liberation of acetylcholine is rapidly accumulating and has been reviewed recently by Gaddum (1936). In particular curare appears not to affect the liberation of acetylcholine in curarized muscles (Dale, Feldberg and Vogt, 1936) and consequently its action must be regarded as antagonistic to that of the normal transmitter. The transmitter is still liberated by nerve impulses but is prevented from having its normal effect. Incidentally this result provides a new criterion of curariform activity.

Since the active alkaloid of curare is a quaternary ammonium salt, it is reasonable to expect that the curariform action of onium salts in general will be found to depend on an antagonism to the chemical transmitter liberated at the nerve endings. This view of curariform action is too recent to make a detailed discussion of it profitable but it does not seem likely to conflict with the general argument of this article that it is the characteristic ionic properties of onium cations which determine the curariform action. Acetylcholine is itself an onium cation and this fact may account for the peculiar effectiveness of foreign onium cations in blocking the action of the chemical transmitter.

The view that onium salts antagonise acetylcholine at myoneural

[4] For attempts to localise the site of action histologically by the use of curarizing dyestuffs see Führer (1908) and Fulton (1921).

junctions in striated muscle does not necessarily involve the conclusion that they should do so at all cholinergic junctions, but the "nicotine" properties of onium salts, first described by Dale (1914), are interesting in this connection. The stimulating nicotine properties (on sympathetic ganglia, the suprarenal medulla and certain striated muscle fibres) are not shown by onium cations which contain less than three methyl groups attached to the central atom, but the paralysing nicotine action on sympathetic ganglia appears to be a general property of onium salts (Dale, 1914; Burn and Dale, 1914; Hunt and Renshaw, 1925; Hunt, 1926). Brown and Feldberg (1935) have recently shown that curarine chloride shares this property. They found that curarine chloride paralysed the superior cervical ganglion of the cat both to nerve stimulation and to small doses of acetychloline, but it did not affect quantitatively the output of acetylcholine from the ganglion during preganglionic stimulation. This observation suggests that the paralysing nicotine action on sympathetic ganglia may be allied to the curariform action, both actions depending on an antagonism to acetylcholine.

No mention has been made in this discussion of Lapique's theory of curarization. This theory, however, offers no reason why onium cations should produce heterochronism; at most it provides an alternative way of describing the physiological data. Moreover, the theory has been recently reviewed and criticized by Rushton (1935).

REFERENCES

ABDERHALDEN, E. AND F. MÜLLER. Med. Klin. **6**: 883, 1910.

AESCHLIMANN, J. A. AND M. REINERT. J. Pharmacol. **43**: 413, 1931.

BOEHM, R. Arch. Pharm. **235**: 660, 1897.
Arch. exper. Path. u. Pharmakol. **63**: 177, 1910.
Heffter. Handb. der Experimentellen Pharmakologie. Berlin. Vol. 2, pp. 179–283, 1920.

BROWN, G. L. AND W. FELDBERG. J. Physiol. **86**: 10 P., 1935.

BRUNTON, T. L. AND J. T. CASH. Phil. Trans. 197, 1884.

BURN, J. H. AND H. H. DALE. J. Pharmacol. **6**: 417, 1914.

CLARK, A. J. The mode of action of drugs on cells. London, 1933.

COWAN, S. L. AND H. R. ING. J. Physiol. **79**: 75, 1933.
Ibid. **82**: 432, 1934.
Ibid. **84**: 90, 1935.

COWAN, S. L. AND W. G. WALTER. Oral communication to the Physiological Society, Cambridge, July, 1935.

CRUM BROWN, A. AND T. R. FRASER. Trans. Roy. Soc. Edinburg **25**: 151, 693, 1868–69.
Proc. Roy. Soc. Edinburg **7**: 663, 1872.

CUSHNY, A. R. J. Pharmacol. **15**: 126, 1920.

DALE, H. H. Ibid. **6**: 147, 1914.

DALE, H. H., W. FELDBERG AND M. VOGT. J. Physiol. **86**: 353, 1936.

EHRLICH, P. AND A. EINHORN. Ber. Deutsch. chem. Ges. **72**: 1870, 1894.
FRÄNKEL, S. Ergebn. Physiol. **3**: I, 290, 1904.
FROMHERZ, H. J. Physiol. **79**: 67, 1933.
FÜHNER, H. Arch. exper. Path. u. Pharmakol. **58**: 1, 1907.
Ibid. **59**: 161, 1908.
FULTON, J. F. Am. J. Physiol. **57**: 153, 1921.
GADDUM, J. H. Gefässerweiternde Stoffe der Gewebe. Leipzig, 1936.
GARTEN, S. Arch. exper. Path. u. Pharmakol. **68**: 243, 1912.
GASKELL, J. F. Phil. Trans. **205B**: 153, 1914.
GOTTLIEB. Published by C. Hartmann and V. Meyer. Ber. Deutsch. chem. Ges. **27**: 1592, 1894.
GRAHAM, H. T. AND H. S. GASSER. J. Pharmacol. **43**: 163, 1931.
HARVEY, E. N. J. Exper. Zool. **10**: 507, 1911.
HILDEBRANDT, H. Arch. exper. Path. u. Pharmakol. **53**: 76, 1905.
HÖBER, R. AND H. WALDENBURG. Pflüger's Arch. **126**: 337, 1909.
HUNT, R. AND R. R. RENSHAW. J. Pharmacol. **25**: 315, 1925.
HUNT, R. Ibid. **28**: 367, 1926.
ING, H. R. AND W. M. WRIGHT. Proc. Roy. Soc. **109B**: 337, 1931.
Ibid. **114B**: 48, 1933.
ISSEKUTZ, B.v. Ztschr. exper. Path. Therap. **19**: 99, 1917.
JOLYET, F. AND A. CAHOURS. C. R. Acad. Sci. Paris **67**: 904, 1868.
KATZ, B. J. Physiol. **86**: 14P., 1935.
KING, H. J. Chem. Soc. London, 1381, 1935.
KULZ, F. Pflüger's Arch. **195**: 623, 1922.
LAIDLAW, P. P. J. Pharmacol. **4**: 461, 1913.
LANGLEY, J. N. Proc. Roy. Soc. **78B**: 170, 1906.
LINDEMANN, W. Arch. exper. Path. u. Pharmakol. **41**: 191, 1898.
LOEB, J. AND W. F. EWALD. J. Biol. Chem. **25**: 377, 1916.
MARSHALL, C. R. Trans. Roy. Soc. Edinburg **50**: 17, 379, 481, 1914–1916.
MEYER, H. Ergebn. Physiol. **1**: II, 199, 1902.
OVERTON, E. Ztschr. physikalische Chem. **22**: 189, 1897.
PAK, C. AND B. E. READ. Chinese J. Physiol. **9**: 1, 1935.
PAL, J. Zentralbl. Physiol. **24**: 1, 1910.
PEISER, F. Pharmakologische Untersuchungen über die Wirkung von Tetra--methyl-, -äthyl- und -propylammonium Salzen. Dissertation. Königsberg (unpublished) 1920.
POHL, J. Arch. intern. Pharmacodyn. Therap. **13**: 479, 1904.
RABATEAU, M. C. R. Soc. Biol. Paris **34**: 127, 195, 1882.
ROTHBERGER, C. J. Pflüger's Arch. **92**: 424, 1902.
RUSHTON, W. A. H. Biol. Rev. **10**: 1, 1935.
SANTESSON, C. G. AND G. KORAEN. Skand. Arch. Physiol. **10**: 225, 1900.
STAHLSCHMIDT, C. Poggendorf's Annalen **108**: 513, 1859.
STRAUB, W. Pflüger's Arch. **79**: 379, 1910.
STEDMAN, E. Biochem. J. **20**: 719, 1926.
Ibid. **23**: 17, 1929.
TRENDELENBURG, P. Heffter. Handb. der Experimentellen Pharmakologie. Berlin. Vol. I, pp. 564–639, 1923.
VULPIAN, A. Arch. Physiol. norm. Path. **1**: 474, 1868.

THE CEREBRAL CIRCULATION

H. G. WOLFF

The New York Hospital and the Department of Medicine, Cornell University Medical College, New York

Munro (1783), in the eighteenth century, formulated an hypothesis which has colored all subsequent inquiry into cerebral circulation. "As the substance of the brain, like that of the other solids of our body is nearly incompressible the quantity of blood within the head must be the same, or very nearly the same, at all times whether in health or disease, in life or after death, those cases only excepted in which water or other matter is effused or secreted from the blood vessels; for in these, a quantity of blood equal in bulk to the effused matter will be pressed out of the cranium."

This thesis, further developed by Kellie (1824) and subsequently known as the "Munro-Kellie Doctrine," was soon widely accepted and, although modified by later researches (Burrows, 1846), has been in the main confirmed (Weed, 1922, 1929 and 1933; Flexner 1934).

However, an unfortunate adherence to the "letter" of Munro's verbalism that the brain is enclosed in a "case of bone" may have delayed interest in the physiology of the cerebral blood vessels, since it has been commonly assumed that they were quite passive and were capable of but little change in diameter (Bayliss and Hill, 1895). Renewed curiosity and technical advance have revealed that the brain vessels actively change in size. This change though not of a major degree, is significant and thus the vessels may vary independently of the systemic blood pressures.

Since the brain is contained in a closed chamber it must be a chamber with an expansile window. This window may be thought of as partially mechanical, permitting the total volume to change by means of plastic surfaces, as for example the inward collapse of dura mater and the volume changes in intradural veins. On the other hand, it is also of a functional nature in the sense that resorption or formation of cerebrospinal fluid may parallel the change in volume of the blood vessels (Weed and Flexner, 1932; Weed, Flexner and Clark, 1932; Flexner, Clark and Weed, 1932).

In brief, it is well to look upon the brain container as an almost rigid box which allows of no major increase in its contents, but which is plastic enough to permit of physiological variation in the amount of cerebral blood.

As a basis for subsequent physiological considerations it is expedient to review the anatomic arrangements of the cerebral vasculature. The blood vessels of the brain differ in no histological essential from vessels elsewhere, though there are minor differences. In the cerebral arteries the elastic fibers are more numerous and have a different arrangement than in other parts of the body (Cobb and Blain, 1933). Furthermore, the cerebral veins have, in relation to their large lumen, extremely thin walls, composed mainly of connective tissue with no uniform number of layers. Muscle fibers are present in the bigger superficial veins but are few in number and hence are frequently overlooked. Also, in most of the deep veins, muscle fibers are seen though again they are not numerous (Stransky and Löwy, 1912–13). Myelinated and unmyelinated nerves and endings traverse the surface of the cerebral vessels in no unusual way.

The cerebral arterial tree, unlike that of many organs, has no hilus from which the vessels plunge into the body of the structure. On the contrary, the abundant resources of blood flowing to the brain through the internal carotid and vertebral arteries are united by the "Circle of Willis", which with its six large branches, encircles the globoid hemispheres at their base. These six great trunks then divide into branches. A few enter the basal ganglia and choroid plexus, but for the most part they spread themselves like a net in finer and finer branches over the surface of the cortex. Smaller arteries at innumerable points dive deeply into the cortical and subcortical tissues where they anastomose with one another and with others coming through the brain substance from the opposite surface of the hemisphere (Cobb, 1932).

The cerebral veins are divided into two groups, the internal and external, with incomplete anastomoses between them. The internal group drains through the great cerebral vein of Galen, running back directly over the pineal body (Bailey, 1934). The external veins emanate from the region of the insula. Because with growth there is anterior displacement of the frontal lobe, and posterior development of the main mass of the hemispheres, the direction of the terminal portion of the great veins is altered; the anterior veins are thus directed posteriorly and the posterior veins course obliquely anteriorly as they pass to the superior sagittal sinus (Stopford, 1930; O'Connell, 1934). The large

venous sinuses drain into channels at the base of the skull. The blood then flows from the cranial cavity like fluid from a flask with a gradually tapering neck.

Since, then, the pial arteries are figuratively the floodgates of the cerebral circulation, it is reasonable to infer that alterations in their caliber directly influence the cerebral circulation. These pial arteries give rise to smaller vessels forming different patterns, depending on whether they supply the neo- or paleo-cortex. In the neopallium the smaller arteries, after leaving the surface, give off a few capillaries in lamina I, and many more in laminae II, III, IV, and V, beneath which lamina VI is again less vascular. The white matter everywhere is relatively less vascular than the gray (Cobb, 1929; Craigie, 1920 and 1921). In the fiber tracts, the arterioles and venules roughly parallel the fibers, so that there is a linear pattern of vessels, the capillary net crossing between, making many small irregular quadrangles (Cobb, 1932).

Similarly, the cerebellum receives its blood from the pial arteries over its convexity. The smaller arteries penetrate into the molecular layer and pass through it, giving off a few capillaries which make large loops. In the granular layer, however, they give off many more capillaries and the loops are smaller and more rounded. Hence, when a thick section is examined with low power magnification a dense weave of capillary net-work appears as a dark band corresponding in position with this cellular layer (Cobb, 1932).

The basal ganglia, being relatively centrally placed, have arteries which enter from all surfaces and penetrate toward the center of the cell mass, subdividing into finer branches. The capillary network forms a pattern of uneven rounded loops which under magnification have the appearance of finely spun nets drawn over the surface of transparent spheres. The capillary bed forming the net appears as a continuous anastomosis (Cobb and Talbot, 1927; Cobb, 1932).

Thus, from a study of the cerebral cortex and basal ganglia in the lower vertebrates and in man there is no evidence for the existence of end arteries (Pfeifer, 1928; Cobb, 1931 and 1932).

In the spinal cord the circulatory mechanism, although analogous to that seen in the brain, differs because of the relative position of the white matter which, lying outside the gray, causes the arterial arrangement to be reversed. As in the brain the gray matter has a richer blood supply than the white matter. The larger arteries lying in the sub-arachnoid space, commonly three in number, send branches through the white matter to the gray beneath. The surface arteries and their perforating

branches thus supply the blood to the spinal cord. No vessels are found within the central canal. There is less anastomosis between the different segments of the cord than between the different parts of the brain, although free anastomosis takes place between capillaries here as in the brain (Cobb, 1932).

Cerebral blood vessels—physical characteristics. The pial arteries when inspected on the surface of the cortex through a tightly closed skull window (Forbes, 1928) have a solid, ribbon-like appearance. No movement of the brain surface is visible. Photographs with magnification of 200 times and exposed for two minutes show no blurring. However, the larger arteries, wherever they change the direction of their course, throb with each cardiac systole, bending and straightening out slightly while gently tugging the arachnoid. The smaller peripheral arterial twigs jerk less and no movement is observed in the smallest. The bright scarlet color of the arteries contrasts with the purplish red of the veins.

The arterioles send off branches more nearly at right angles than do the venules. Some of the arterial branches have an annular constriction at their point of origin from a larger artery. As a consequence that portion of the artery immediately peripheral to the junction is of greater diameter (Florey, 1925). Annular constrictions may appear in an exaggerated form when there is vasodilatation after mechanical trauma (Hertzman and Franke, 1935).

The movement of blood corpuscles in the lumen of the arteries and arterioles cannot be discerned when the blood pressure is average. However, when the systemic arterial pressure falls to 15–20 mm. of mercury the movement of the individual red blood corpuscles can be seen (Wolff). This continues until death when, if the chest is slightly lower than the head, reversal in the direction of flow occurs and the cerebral arteries usually become empty. In the minute vessels and veins (Forbes, 1928) contrary to the arteries, the movement of the corpuscles, although rapid, is normally slow enough to be readily visible. The flow in the minute vessels is pulsatile. In the slightly larger venules the flow may appear slower and steadier, while in the largest tributary veins it becomes increasingly more rapid and may become so rapid as to be no longer visible.

The movement of the corpuscles within the veins is influenced by respiration. With deep respiratory movements the venous flow alternately slows and speeds up. The change in speed of flow is not synchronous with the beginning or end of either phase of respiration, but

starts after the middle of expiration and ends after the middle of inspiration.

The flow in the minute vessels is remarkably constant under ordinary conditions and these vessels do not periodically appear and disappear. The number of red cells in them may change from time to time but unless the blood pressure falls appreciably these vessels do not empty completely. Whether periodic opening and closing of the minute vessels within the substance of the brain occurs has not been satisfactorily determined.

The diameter of the cerebral arteries, although constant for as long as an hour, may change spontaneously from time to time (Forbes and Wolff, 1928). Similarly, although the speed of flow is relatively constant, sudden change of position or blood pressure may be followed by a change of circulation. [This has been determined for the head by a stromuhr in the common carotid (Burton-Opitz and Edwards, 1916). The intracranial blood flow has been determined by the following methods: outflow from the cerebral veins (Howell, 1898); time for radium emanation to travel from the internal carotid artery to the internal jugular vein (Wolff and Blumgart, 1929); thermocouple in the internal jugular vein (Gibbs, 1935). The cerebral blood flow has been measured by a thermocouple in the brain substance (Wolff and Cattell, 1935).] Variations in systemic venous pressure within rather wide limits (11 to 80 mm. Ringer solution) produces no change in the caliber of the pial arteries (Finley and Forbes, 1933).

Electrical stimulation applied directly to the arterial wall causes vigorous vasoconstriction and is the only type of stimulation which produces enough change in the lumen to occlude the cerebral arteries completely or almost completely. The vasoconstriction may be present from 15 to 20 seconds to 2½ hours after cessation of stimulation (Florey, 1925). Ice applied to the cortex for 15 to 30 seconds causes moderate contraction whereas temperatures of 40°C. may result in dilatation. Higher temperatures (45°–47°) are said again to cause contraction but inconstant and variable results are obtained with temperatures about 40°C.

Cortical blanching can be locally produced by increasing intra-cranial pressure. This was done by forcibly injecting saline through a syringe attached to a skull window. This local cerebral anemia is followed by vasodilatation. That the compression effect was not generalized was shown by the fact that during the injection there was no appreciable increase in intracranial pressure as measured by a manometer needle in

the cisterna magna. As with local cerebral anemia, cerebral asphyxia produced by causing the animal to breathe various carbon monoxide and air mixtures always results in maximal long-lasting cerebral vasodilatation. In fact, asphyxia produced by whatever means is always followed by cerebral vasodilatation, assuming that the blood pressure is not too low (Wolff).

Before entering more deeply into the consideration of cerebral circulation it is well to appreciate that many erroneous conclusions have arisen from inadequate methods of observation (Forbes and Wolff, 1928). Recent descriptions of the free anatomoses between the internal and external carotid arteries (Bouckaert and Heymans, 1935) particularly in the dog and cat, demonstrate that internal carotid artery flow, often taken as an indicator of intracranial blood flow is not a measure of cerebral blood flow alone. Hence, as an index of cerebral circulation, the extensive data on changes in pressure, temperature and stream volume in the internal carotid artery must be viewed with caution, even though they may not be completely waived.

Less difficult to interpret are the direct micrometric observations of superficial or deeper brain vessels (Forbes, 1928) though no general conclusions may be drawn concerning blood vessels in all parts of the brain from such limited fields of observation. On the other hand, accurate measurement of temperature variation of the brain substance is the most reliable index of local cerebral blood flow (modification by Schmidt and Pierson, (1934) of a method developed by Gibbs (1933)). The selection and evaluation of data throughout the subsequent discussions have been influenced by these general criticisms.

Cerebral circulation and the action of chemical agents. Since chemical agents have been instrumental in shaping concepts concerning the brain circulation, it is relevant to consider here several of these substances in more detail.

The effect of epinephrine on the cerebral vasculature has frequently been studied (Forbes and Wolff, 1928; Forbes, Finley and Nason, 1933; Riser and Sorel, 1930; Riser, Meriel and Planques, 1931). Observations have not always been in accord, but recent experiments explain apparently conflicting results. Thus, when epinephrin (in dilutions of 1:10,0000 to 1:500,000) was applied locally to the pia over the cortex (cats and monkeys) vasoconstriction of pial arteries was observed, the average constriction being about 18 per cent. Intravenous injection, however, caused a rise in systemic blood pressure, and pial artery widening in more than half the experiments. Sometimes there was a slight

constriction after the blood pressure effect was over and when the blood pressure had reached its initial level. Also, the cerebral blood flow, as determined by a thermocouple in the brain substance, at first increased, the amount depending on the dosage and the rise in systemic pressure. This was sometimes followed by decrease in flow (Schmidt, 1934; Wolff and Cattell, 1935). Intracarotid injection caused increase in constriction in most of the remainder, again not due to a fall in blood pressure.

On the other hand, when the blood pressure was kept constant by means of an artificial perfusion system (Finesinger and Putnam, 1933) epinephrine in the perfusion fluid caused a significant decrease in cerebral blood flow (30 to 40 per cent in monkeys and cats). Under these circumstances direct observations of pial vessels revealed vaso constriction averaging about 10 per cent (in cats).

In other words, epinephrine, when applied locally to the surface of the cerebral cortex, usually causes vasoconstriction; in contrast, when injected intravenously there is usually widening of the cerebral arteries during the subsequent rise in systemic arterial pressure. The increase in diameter is sometimes followed by vasoconstriction. Similar effects are observed after intracarotid injection. Thus, the local vasoconstrictor effect of epinephrine may be overcome by the rise in systemic arterial pressure caused by the action of epinephrine. When the systemic arterial pressure is kept artificially constant, epinephrine in the blood stream then causes cerebral vasoconstriction only. The effect of any given amount of epinephrine is the resultant of two opposing forces—a weak local constrictor action and the relatively stronger expansile action of the raised systemic arterial pressure.

Because the effects of acetylcholine closely resemble those of the cholinergic neuro-humoral agent, its effect on the cerebral vessels is of interest. Acetylcholine, when given intravenously, caused pial vasodilatation averaging 22 per cent with a blood pressure fall of 69 per cent (Wolff, 1929). As with histamine and amyl nitrite, cerebral vasodilatation can occur in spite of a fall in systemic blood pressure. Despite the cerebral vasodilatation the cerebral blood flow, (as determined by a thermocouple in the brain substance, Wolff and Cattell, 1935) may actually be slower during the fall in blood pressure. After the blood pressure begins to return to its original level, the blood flow considerably increases for some time (Wolff and Cattell, 1935; also, Schneider and Schneider, 1934, using a thermocouple in the internal carotid).

The effects of histamine demonstrate the inadequacy of even maximal cerebral dilatation in maintaining cerebral blood flow if systemic arterial

blood pressure falls too low. Histamine causes dilatation of all visible blood vessels in the pia: arteries 288 micra in diameter and vessels of capillary size are alike affected (animals under barbiturate anesthesia). The diameter of the vessels may increase as much as 50 per cent. After intravenous injection, the cerebral vascular dilatation is accompanied by a fall in systemic arterial and a rise in systemic venous pressure and in cerebro-spinal fluid pressure. Occasionally, with a great fall in blood pressure, a slight transient narrowing of the pial vessels immediately precedes the dilatation (Forbes, Wolff and Cobb, 1929).

In cats anesthetized with ether, however, the pial arteries may become narrower after intravenous histamine. Thus, a discrepancy in reported observations on the effect of histamine on cerebral vessels resolves itself into whether ether or a barbiturate is used as an anesthetic. If ether is used, the cerebral vessels become moderately dilated; histamine, because of its action of lowering the blood pressure, narrows the already dilated vessels, and its own vasodilator effect is thus not discernible. In contrast, after barbiturate anesthesia, histamine produces only cerebral vasodilatation. Ether probably has no specific cerebral dilator properties not possessed by the barbiturates. It is possible, however, that in the initial stages of ether anesthesia in animals, the depth of anesthesia and carbon dioxide output are less readily controlled, and greater cerebral vasodilatation may thus incidentally accrue.

After histamine injection the volume of blood passing through the internal carotid falls off rapidly but soon begins to increase, eventually exceeding its original volume during the slow return of the blood pressure to normal (Schneider and Schneider, 1934; C. J. Keller, 1930, with a modification of the Rein thermostromuhr on the internal carotid). The secondary increase in cerebral blood flow is the result of cerebral vasodilatation. Continuous infusion of histamine demonstrates, however, the dependence of cerebral blood flow on blood pressure despite maximal cerebral vasodilatation (Wolff and Cattell, 1935). When dilute solutions of histamine are slowly infused intravenously, the systemic arterial blood pressure falls, and the cerebral blood flow becomes slower (determined by a thermocouple in the brain substance). The blood pressure and the cerebral blood flow remain at a lower level as long as the perfusion continues. With cessation of the perfusion, the cerebral blood flow increases, and reaches a level considerably higher than before the histamine infusion. In other words, the cerebral vasodilator action of histamine is without effect in increasing the cerebral blood flow until the blood pressure level has almost returned to what it was before the

drug was injected. Also, if one histamine injection is followed by a second, given just before the systemic arterial blood pressure has regained its original level, the increase in cerebral blood flow that usually accompanies this phase of the histamine action does not occur. The cerebral blood flow is again slowed, and reaches the low ebb that it had shortly after the first injection.

If at the height of the increased cerebral blood flow following histamine injection the blood pressure is caused to fall by increasing the intrathoracic pressure through distention of the lungs, then the cerebral flow, in spite of the vasodilator action of the histamine, is promptly decreased. With the return of intrathoracic pressure to normal the cerebral blood flow again reaches its augmented level, thus to remain for the usual time (Wolff and Cattell, 1935).

In brief, despite an increase in the diameter of the cerebral vessels as great as 50 per cent, the brain blood flow may decrease if there is a considerable fall in blood pressure. Furthermore, these experiments demonstrate the difficulty and probable error in drawing inferences concerning the diameter of brain vessels from blood flow values alone (Wolff and Cattell, 1935).

Pituitary posterior lobe extracts, according to some (Howe and McKinley, 1927; Florey, 1925), produce no constriction of pial vessels. Others have observed both constriction and dilatation (Wolff, 1929). Thus, in cats, (Forbes, Finley and Nason, 1933), posterior lobe extracts ("pituitrin" and "pitressin") when applied locally under skull windows produced inconstant effects, widening of the arteries occurring in more than half of the experiments. When given intravenously the results were again inconstant, increase in diameter also occurring in more than half of the experiments. Usually when the systemic arterial pressure rose appreciably the cerebral arteries at first increased in diameter, then, shortly after the peak of the rise or when the pressure was falling, the pial arteries became narrower, and in about 8 per cent the diameter became less than that preceding injection, independently of blood pressure changes. Initial vasoconstriction approximating 8 per cent occurred in about a fifth of the experiments.

Observations on the cerebral blood flow as measured by a thermocouple in the internal carotid of dogs (Schneider and Schneider, 1934) reveal that following "hypophysin" there is a decrease in cerebral blood flow which continues as long as the blood pressure remains high. Thus, it would seem that the cerebral vasoconstriction is of a degree which is not overcome by the moderate elevation of the systemic arterial blood

pressure. It is to be recalled, however, that because of anastomoses between the internal carotids no definite conclusions concerning blood flow determined by this method are justified.

It would seem that the posterior pituitary lobe extract may both increase and decrease diameter of cerebral arteries, depending to some extent on the method of administration and the systemic arterial blood pressure, but to a greater degree on unknown factors. The cerebral vasomotor reaction to the gland substance would, at best, appear to be a minor one, but qualitatively, perhaps not unlike that of many other vascular beds.

There is general agreement concerning the effect of amyl nitrite and other nitrites on the blood vessels of the brain (Leake, Loevenhart and Muehlberger, 1927; Florey, 1925; Wolff, 1929; Schneider and Schneider, 1934). When amyl nitrite is inhaled, the blood pressure falls and the pial arteries, veins, and minute vessels dilate.

The action of caffeine on the cerebral vessels is similar to the vascular response to caffeine in other parts of the body (Finesinger, 1932). During amytal anesthesia the intravenous injection of caffeine in cats caused an average increase in diameter in the pial arteries of 29 per cent (by skull window observations). There was an abrupt drop in blood pressure with immediate return to normal. Cerebral blood flow was then increased (as measured by a thermocouple in the internal carotid, Schneider and Schneider, 1934). However, during ether anesthesia, intravenous injection of caffeine caused a transient narrowing, averaging 18 per cent, during an abrupt drop in blood pressure. As was noted in histamine studies, the cerebral vasodilator effect of caffeine was masked since the cerebral vessels were already dilated by ether.

Hypertonic solutions, when injected intravenously or intraperitoneally cause, after short-lived increase in diameter, moderate constriction of the pial arteries and arterioles (by skull window observations, Wolff and Forbes, 1928). Later the arteries, the minute vessels and veins widen (Kubie and Hetler, 1928; by skull window observations, Forbes and Nason, 1935) and the blood volume of the brain increases (as determined by adding silver proteinate to the blood and ashing, Weil, Zeiss and Cleveland, 1931), due presumably to the well marked decrease in intracranial pressure and to the elevation of the systemic arterial pressure. With the elevation in blood pressure the rate of cerebral blood flow is at first increased despite the slight constriction of the larger vessels (thermocouple in the internal carotid of dogs, Schneider and Schneider, 1934).

It is not relevant here to discuss the reaction of every chemical agent

on the cerebral vasculature, but a few miscellaneous observations of interest may be mentioned. Most anesthetics cause some cerebral vasodilatation. Ether causes more, the barbiturates less (Schmidt and Pierson, 1934; Finesinger and Cobb, 1935). When the anesthesia is very deep the effect of asphyxia adds to the vasodilator effect of the anesthetic. Barium chloride solution (5 per cent) causes marked constriction of cerebral and medullary vessels (Florey, 1925). Hydrogen sulfide in relatively large amounts acts on the cerebral circulation in a manner similar to other acids, causing dilatation of arteries in the pia. In smaller amounts the gas often causes a slight constriction (Forbes and Krumbhaar, 1933).

The results of ergotamine tartrate injection on pial vessels are inconstant: dilatation, constriction, or no change occur equally often (by skull window observations, Pool and Nason, 1935). The changes in cerebral flow are, in almost all cases, in the same direction as the changes in blood pressure (as determined by a thermocouple in the internal jugular vein, Lennox, Gibbs and Gibbs, 1935). But in contrast (Pool and Nason, 1935) dural vessels constricted on the average 25 per cent and skin vessels 39 per cent.

The convulsions produced by absinthe, homocamfin, monobromated camphor, and picrotoxin, as well as those occurring spontaneously in man, are apparently not associated with significant or consistent changes in the cerebral circulation (by skull window observations in cats, Finesinger and Cobb, 1933; by a thermocouple in the internal jugular vein in man, Gibbs, Lennox and Gibbs, 1934).

In brief, basic principles of the brain circulation are illustrated by the action of the common pharmacodynamic agents. In maintaining constant, increasing, or decreasing cerebral flow,—cerebral vasodilatation and constriction, compensate for hydrostatic effects only within a comparatively narrow range of pressure variation. When the blood pressure exceeds this margin, the effect of cerebral vasoconstriction in restricting blood flow is offset; and vice versa, when the range is exceeded in the opposite direction, i.e., with a blood pressure fall, cerebral vasodilatation is inadequate to sustain a constant cerebral blood flow. Thus, epinephrine demonstrates that in so far as the cerebral blood flow is concerned, cerebral vasoconstrictor effects are readily offset by elevations in arterial blood pressure. By histamine it is shown that a constant or even adequate cerebral blood flow is not maintained despite maximal cerebral vasodilatation, if there is a coincident and appreciable fall in blood pressure.

Finally, even more important than their rôle as illustrators of fundamental principles, is the influence that one of these substances actually exerts in the regulation of the cerebral circulation. It has already been suggested, and it will become more apparent in subsequent discussion that carbon dioxide is an important regulator of cerebral blood flow.

Cerebral circulation and respiration. The influence of the cerebral blood flow upon respiration has been experimentally established (Rosenthal, 1865; Gesell et al., see Schmidt, 1928), although recent observations according recognition of the carotid sinus as a potential respiratory regulator have revealed that some of the effects previously attributed to cerebral blood flow alone are due in part to carotid sinus action (Heymans, Bouckaert and Regniers, 1933). There is, however, a difference of opinion concerning the relative magnitude and physiological importance of these reflex effects.

That respiration may be directly affected by alteration in the cerebral blood flow is deduced from the following evidence (Hill, 1896; Schmidt, 1932[II]). First, if the cerebral blood flow is increased by adrenalin, apnoea occurs in decerebrate animals even after section of sinus and aortic nerves, which indicates that the responsible factors must be localised in the brain stem. Secondly, studies of the effects of variation in cerebral blood flow show that both with and without carotid sinus nerves (assuming that the carbon dioxide content of inspired air remains constant), respiratory depression follows increase in cerebral blood flow, whereas increased respiratory activity follows a decrease in blood flow. Thirdly, the respiratory excitant effect of sudden cerebral anemia can overcome the strongest reflex inhibition resulting from high endosinusal pressure. Fourthly, in experiments in which the cerebral blood vessels are artificially perfused, respiratory depression followed increases in cerebral blood flow as slight as 6 to 13 per cent of the preceding flow. In contrast, respiratory depression did not occur until the endosinusal pressure was increased from 12 to 23 per cent. From these data it was concluded (Schmidt, 1932[II]) that the respiratory effects of alterations in cephalic blood pressure may be due either to reflexes or to alteration in cerebral blood flow or to both. Also, it was inferred that the influence of cerebral blood flow may be as sensitive as the reflex effect and more powerful, although the sensitivity of the central effect is more variable. And finally, the sinus reflex influence is not essential to the regulation of breathing, except in the hyperpnea of anoxemia which appears to be mainly, if not entirely, reflex in origin.

The Ghent school, however, attributes greater influence to the carotid

sinus as a regulator of respiration. For example, Bouckaert and Heymans (1933) find that occlusion of both vertebral arteries provokes an immediate and abrupt fall in cerebral blood pressure of from 80 to 40 mm. of mercury. But in spite of this fall, the general blood pressure and respiration remain unaffected. Neither does the subsequent sudden release of the vertebral artery influence the vasomotor or respiratory function. In contrast, a decrease in the pressure within the isolated carotid sinus greatly affects the respiratory rate in spite of an actual increase in cerebral blood flow. Schmidt (1932[II]) confirmed this observation, but suggests that the drop in cerebral blood flow resulting from vertebral occlusion, which he calculates to be about 25 per cent, may be insufficient to produce a change in respiration. Bouckaert and Heymans indicate that if cerebral blood flow must drop more than 25 per cent before a change in respiration occurs, the cerebral blood flow mechanism cannot be of prime physiological significance. Furthermore according to their observations, hyperpnea provoked by hypotension in the carotid sinus zone persists in the face of an alkalosis actually brought about by the hyperpnea and, vice versa, apnea due to hypertension in the sinus zone persists in the face of hypercapnea of the blood perfused through the central respiratory mechanism.

Thus it is probable that the aortic and sinus reflexes have a potentially significant regulatory function, but whether these reflexes are more or less important than the cerebral blood flow in this regard is uncertain. Definite conclusions concerning the relative importance of central versus peripheral respiratory regulation cannot as yet be formulated.

Let us turn from the less basic question of the relative preeminence of the two mechanisms and consider the more vital issue, i.e., the interplay between the systemic arterial pressure, the diameter of the cerebral vessels and the volume and rate of respiration.

With hyperventilation there is a slight fall in systemic arterial pressure and cerebral vasoconstriction. Also with spontaneous constriction of cerebral vessels there is augmentation of respiration followed by respiratory depression (Schmidt, 1928,[III]; Wolff and Lennox, 1930). With moderate increase in cerebral blood flow, induced by artificially raising the circulatory pressure, constriction of brain vessels often follows. Inversely, when the cerebral flow is decreased by lowering the perfusion pressure the cerebral vessels dilate. Also, the respiratory minute volume decreases as the carotid increases and vice versa, as the flow decreases the volume increases (by the thermoelectric method of Gesell and Bronk (1926) in the carotid and femoral arteries, Greeley

and Greeley, 1928). In other words, the respiration usually varies inversely with the cerebral blood flow. The effects are not dependent on the changes in pressure in the carotid sinus or the integrity of the vagus or depressor nerves (Schmidt, 1932[II]).

In a physiological emergency as, for example, if the inspired air be rich in carbon dioxide and poor in oxygen, all possible mechanisms for the maintenance of adequate cerebral oxygen supply are thrown into action, i.e., the minute volume of the inspired air increases, the systemic arterial blood pressure becomes elevated and the brain vessels dilate (by skull window observations, Wolff and Lennox, 1930), and the cerebral blood flow increases (as determined by a thermocouple in the brain substance, Wolff and Cattell, 1935).

The significance of blood pressure effects is observed when, for one reason or another, carbon dioxide fails to raise the blood pressure, although it causes dilatation of cerebral vessels. The usual augmented respiration may fail to occur and the reverse, i.e., apnea may result. It has consequently been inferred that, in the absence of the vasomotor response, the ability of the respiratory mechanism to react to stimuli such as carbon dioxide may be limited (Schmidt, 1928[III]). In addition, as has been suggested by Schmidt, the vasomotor responses, because of the rapidity of their effects, restore breathing to normal more satisfactorily than the cerebrovascular mechanisms. These blood pressure responses diminish respiratory irregularities.

An increase in the carbon dioxide content of the respired gas induces prompt change in respiration before either the systemic arterial blood pressure or the size of the pial arteries is altered. However, as regards the relative effects of carbon dioxide increase and oxygen lack, there is a stricter parallelism in the behaviour of the cerebral vessels and respiration. Thus, just as the air containing an increased amount of carbon dioxide is a much more active stimulator of respiration than is air poor in oxygen (Haldane, 1927), so a small rise in carbon dioxide appreciably increases the diameter of cerebral vessels whereas a considerable fall in the amount of oxygen is necessary to obtain the same result.

Cerebral circulation—vasomotor phenomena. The subject of cerebral vasomotor function and structure has been adequately reviewed (Hürthle, 1927; Forbes and Wolff, 1928; Hassin, 1929; Stoehr, 1928; Penfield, 1932; Chorobski and Penfield, 1932; Clark, 1934). More recent data and correlations will be considered here.

Intracranial blood vessels are supplied by both myelinated and un-

myelinated nerves and endings. But few ganglion cells are observed along the vessels of the central nervous system, although peripheral nerve fibers may be traced along the vessels of the medulla, pons, mesencephalon, diencephalon, cortex and the vessels of the pia and choroid plexus (Penfield, 1932; Clark, 1934). From histological studies no conclusion may be drawn as to the possible motor or sensory function of such nerves or endings. Physiological studies, however, indicate that in addition to efferent there are afferent fibers (Levine and Wolff, 1932; Pickering, 1933; Clark, Hough and Wolff, 1935).

Some vasoconstrictor nerve fibers pass to the cerebral vessels via the cervical sympathetic nerves. Also, some vasodilator nerves pass to the cerebral vessels from the medulla oblongata via the facial nerve and through the great superficial petrosal nerve. A direct tract of nerve fibers consisting of myelinated and unmyelinated nerves joins the facial and greater superficial petrosal nerves. Moreover, there is a tract of similar nerve fibers which connects the greater superficial petrosal nerve with the pericarotid plexus (Chorobski and Penfield, 1932).

The effects of cervical sympathetic stimulation on the cerebral vasculature have been thoroughly explored by various methods, and in essence the results agree. Direct observations were made through skull windows in cats, anesthetized (Forbes and Wolff, 1928) and unanesthetized (Thomas, 1935) as well as in the isolated perfused head (Pool, Forbes and Nason, 1934). The average constriction resulting from such stimulation is about 8 to 10 per cent. Experiments carried out by means of blood flow determination methods such as a constant pressure perfusion pump (Finesinger and Putnam, 1933), a thermocouple on the internal carotid (Gollwitzer-Meier and Eckardt, 1935; Schneider and Schneider, 1934), or a thermocouple in the substance of the brain (Schmidt and Pierson, 1934; Schmidt, 1934 and 1935; Wolff and Cattell, 1935) reveal that stimulation of the cervical sympathetic nerves causes decrease in cerebral blood flow and constriction of the pial and intrinsic blood vessels of the cerebrum. As ascertained by blood flow methods, using a thermocouple in the brain substance (Schmidt, 1935), and a Rein thermostromuhr on the internal carotid, common carotid and vertebral arteries, (Gollwitzer-Meier and Eckardt, 1935) the effects are said to be bilateral; by the skull window method, and by actual measurement of capillary filling in both cerebral hemispheres (Talbot, Wolff and Cobb, 1929) the effects are shown to be distinctly unilateral. Moreover, stimulation of the cervical sympathetic nerve causes constriction of the dural arter-

ies, averaging 34 per cent. This contrasts with the average constriction of pial arteries which is 8 to 10 per cent, and the skin which is about 80 per cent (Pool, Nason and Forbes, 1934).[1]

Vasomotor effects are not equally great in all parts of the neuraxis. The blood vessels in the medulla, for instance, are very little affected by stimulation of the cervical sympathetic. The arteries of the hypothalamus are affected somewhat more, and those in the cortex appear to be most affected, although less than are those of the pia (Schmidt, 1934, 1935). The latter in turn have less reaction than the vessels of the dura and skin (Pool, Nason and Forbes, 1934). Stimulation of the cervical sympathetic nerves commonly results in constriction of the arteries of the choroid (by direct observation, Putnam and Ask-Upmark, 1934).

The responses of the pial arteries to faradic stimulation in the thalamus and hypothalamus were also observed through skull windows. Constriction of the pial arteries results from stimulation of a hypothalamic area between the cerebral peduncle and the third ventricle. On the other hand, dilatation of pial arteries results from stimulation in a circumscribed area in the ventral portion of the tuber cinereum as well as from a more diffuse area in the thalamus (Penfield and Stavraky, 1935).

Before discussing the effects of vagus stimulation, it would be well to consider another type of response, said to be reflex in origin, and resulting from occlusion of the common carotid arteries in dogs (Rein, 1929–30). It has been found that when one (the left) common carotid is occluded the systemic arterial blood pressure rises from 10 to 45 per cent, and the blood flow measured by a thermostromuhr on the opposite carotid (i.e., the right) increases 50 to 100 per cent. In the portion of the latter which is beyond the exit of the thyroid artery, the increase in blood flow is 80 to 100 per cent. It has been inferred that the increase in blood flow in the unobstructed carotid is not solely the result of the rise in systemic arterial pressure, but is largely the sequel to reflex vasodilatation in the vascular bed supplied by the common carotid.

This phenomenon was further explored by the Schneiders (1934), who observed that occlusion of the common carotid increases the flow in the

[1] After completion of this review there arrived from Professor Bouckaert a personal communication of recent data on the effect of cervical sympathetic stimulation. It was observed that sympathetic stimulation, by increasing the resistance, raised the pressure in a perfusion system of the completely isolated brain vascular bed. From these observations constriction of the cerebral arteries was inferred. After adrenalin similar effects were observed.

opposite internal carotid artery, sometimes as much as 100 per cent. To ascertain the nature of this response the same experiment was performed on a dead animal. Only minimal increase in flow was produced in isolated perfusion experiments on the dead head, and it was concluded that the increase in flow in the live animal was reflex in nature. According to the Schneiders, the reflex does not appear if the internal maxillary is tied off above the point where the middle meningeal comes off. They infer that in the region of the middle meningeal there is a receptor zone which is stimulated by a fall in pressure, and that from this zone there arise impulses which reflexly lead to increase in blood circulation in the brain vessels.

Bouckaert and Heymans (1935), using Hürthle's method for recording changes of the peripheral resistance in the cephalic end of the internal carotid artery, repeated these experiments and likewise observed that clamping off the external carotid, internal maxillary and ophthalmic arteries resulted in increased flow in the ipsolateral internal carotid. Unlike the Schneiders, these investigators observed no increase in flow when the clamping was limited to the middle meningeal artery; they observed it only when the ophthalmic artery was clamped. They found also that it made but little difference whether the head was dead or alive, and concluded that in the dog the anastomosis of the internal and external carotid arteries through a branch of the ophthalmic artery is responsible for the increased flow in the internal carotid, and that no reflex vasomotor phenomenon is implicated.

Further discussion of this important but uncertain matter will be waived, and the effects of vagus stimulation considered. The results of vagus stimulation are definite and predictable, although interpretation of their nature is more complicated than in the case of the cervical sympathetic effects (Forbes and Wolff, 1928; Cobb and Finesinger, 1932; Ask-Upmark, 1935). With the skull window technique in cats and in monkeys there was observed during the period of vagus stimulation, and while the blood pressure was falling, an average increase of 15 per cent in the diameter of the pial vessels in approximately three-quarters of all experiments. The effects were bilateral. Similar results follow depressor nerve stimulation (Forbes, 1935).

The facial nerve is the aforementioned efferent pathway for this cerebral vasodilator effect (Cobb and Finesinger, 1932; Chorobski and Penfield, 1932), since dilatation of pial arteries is quite regularly obtained on vagus stimulation when the facial nerve is intact, but never when the latter is cut near its exit from the medulla.

The effects of vagus stimulation on cerebral blood flow depend largely,

however, on the concomitant effects on systemic blood pressure. With a constant blood pressure perfusion system, in monkeys and cats vagus stimulation causes a slight, but definite increase (8 per cent) in the volume of cerebral flow during the period of pial vasodilatation (combined perfusion and skull window technic, Finesinger and Putnam, 1933). Moreover, through thermostromuhr measurements on the internal carotid in animals with uncontrolled blood pressure, it has been ascertained that on vagus stimulation there is a relative increase in cerebral blood flow which occurs with the fall in arterial pressure. The increase is bilateral and, as mentioned above, most often not absolute but relative; that is, the flow in the common and internal carotid arteries more quickly approaches the pre-stimulation level than does the level of systemic arterial pressure (Schneider and Schneider, 1934; Gollwitzer-Meier and Eckardt, 1935). Hence, in spite of the cerebral vasodilatation, the cerebral blood flow is seldom absolutely increased during vagus stimulation. In fact Schmidt, using a thermocouple in the brain substance (Gibbs, 1933; Schmidt, 1934; Schmidt and Pierson, 1934; Schmidt, 1935) observed after vagus stimulation no increased blood flow in the parenchyma of the medulla, hypothalamus or parietal cortex of the cat. Also, Wolff and Cattell (1935), employing a similar technic, observed on vagus stimulation that the cerebral blood flow in the parietal cortex slowed in about 80 per cent of their experiments. Moreover, no effect on the cerebral blood flow was observed when vagus stimulation had no effect on the blood pressure. But in an additional 12 per cent, after the initial fall in blood pressure on vagus stimulation with its corresponding decrease in cerebral blood flow, there was an increase in flow to a level higher than that prevailing before stimulation, not to be explained by a rise in blood pressure. Only in about 5 per cent of the experiments was there a slight absolute increase in cerebral blood flow during the fall of systemic arterial blood pressure.

Thus, direct observation of the pial vessels reveals that in most instances when respiratory changes are controlled, the arteries dilate about 15 to 90 seconds after the onset of stimulation of the vagus and depressor nerves. However, an absolute increase of the cerebral blood flow does not follow this stimulation. In fact, if the fall in blood pressure is considerable, the cerebral blood flow is decreased but not in proportion to the fall in blood pressure. In experiments in which the blood pressure is prevented from falling by means of a pericardial blood pressure compensator, the brain blood flow may actually increase during the period of stimulation (Wolff and Cattell, 1935).

A reasonable formulation to be derived from these observations is that cerebral vasodilatation occurs on vagus and depressor nerve stimulation, but that it is not sufficient to increase or even to maintain a constant cerebral blood flow if at the same time there is an appreciable fall in systemic arterial blood pressure.

It is important to consider whether the observed vasodilatation is the direct effect of vagus-depressor impulses on the vasodilator mechanisms via the brain-stem, or whether it is secondary to changes in the blood pressure. Especially to be recalled in this regard is the disputed formulation of the Schneiders, i.e., that a fall in blood pressure in the middle meningeal artery is the stimulus which dilates reflexly the branches of the internal carotid artery.

There is no doubt that maximum dilator effects on vagus stimulation do usually accompany the great falls in blood pressure. In fact, according to Forbes, when the major falls in blood pressure were prevented by clamping the abdominal aorta, dilatation occurred in somewhat less than a quarter of his experiments. However, several other satisfactory experiments (Finesinger and Putnam, 1933) indicated that the vagus-depressor nerve effects are not entirely the result of blood pressure changes. For instance, when the perfusion pressure was kept constant (Finesinger and Putnam, 1933) the cerebral vasodilatation on vagus stimulation still occurred. Moreover, experiments (Cobb and Finesinger, 1932) demonstrated that after cutting the facial nerve which contains the cerebral vasodilator fibers, cerebral vasodilatation on vagus stimulation was not observed. That the fall in blood pressure, though frequent, is not essential for the effect is further demonstrated by the fact that 15 per cent of the vagus vasodilator effects were without a concurrent fall in blood pressure (Forbes and Wolff, 1928). In more recent vagus experiments (Forbes, 1935) vasodilatation occurred in 7 of 15 instances in which there was no fall in blood pressure. These data indicate that the fall in blood pressure, though possibly a factor in the cerebral vasodilatation observed to follow vagus stimulation, is not solely responsible.

It has also been demonstrated that the effects of vagus stimulation occur after resection of the carotid sinus and depressor nerves in the cat (Forbes, 1935). Hence, the effect of the stimulation of the vagus nerve is not dependent upon the presence of the other afferents. Also, resection of the cervical sympathetic nerve does not alter the effect of vagus stimulation.

Furthermore, the cerebral vasodilatation that follows vagus-depressor nerve stimulation is not the result of changes in intrathoracic pressure or venous pressure (Finley and Forbes, 1933). And even though an increase in blood carbon dioxide may augment the effects of vagus stimulation, such effects occur also when the chest is opened and the animal is receiving artificial respiration (Forbes and Wolff, 1928; Forbes, 1935).

It would therefore seem justifiable to conclude that the cerebral vasodilatation that follows experimental vagus and depressor nerve stimulation is, at least in part, the direct result of afferent nerve impulses acting on cerebral vasodilator reflex mechanisms but that there is, in addition, a series of factors operating together to augment this vasomotor effect.

The effects of carotid sinus stimulation are less constant. Ask-Upmark (1935), using skull window observations in cats, found on faradic stimulation of the sinus nerve and by increasing the endosinusal pressure that the blood pressure fell in all instances, and in about 27 per cent the pial vessels dilated with the fall in blood pressure; narrowing occurred in the others. As was the case with vagus stimulation, Schneider and Schneider (1934) observed on carotid sinus stimulation a relative increase in blood flow in the internal carotid artery during the fall in blood pressure (i.e., a fall not as great as would be anticipated from the fall in general pressure).

Bouckaert and Heymans (1933) approached the question of the effect of the carotid sinus on cerebral circulation in the opposite manner, that is, instead of stimulating the sinus nerves, they severed them. As a preliminary experiment, they measured the blood flow in the internal carotid and occipital arteries and in a femoral artery (dogs) by means of a thermo-electric stromuhr (Rein, 1929–30). The experiments showed that with carotid sinus pressure increase (165 mm. Hg) and consequent fall in systemic blood pressure (60 mm. Hg) the cerebral blood flow was reduced. And, vice versa, when the carotid sinus pressure was reduced (20 mm. Hg) and the systemic arterial pressure was elevated (180 mm. Hg) the cerebral blood flow was more than doubled.

Then the aortic and carotid sinus nerves were cut, provoking a general arterial hypertension (180–200 mm. Hg). The systemic blood pressure was measured in the femoral artery. The leg blood vessel tonus was measured by putting a cannula in the peripheral end of the femoral artery. The cerebral vascular tonus was measured by connecting a mercury

manometer with the cephalic end of the internal carotid artery. Acetylcholine (0.0001 mgm.) was then injected into the leg arterial blood stream. This was followed by a prompt dilatation of the blood vessels of the leg. A larger dose (0.002 mgm.) was injected into the internal carotid artery toward the cerebral circulation. No relaxation of the cerebral vasculature was ever recorded. Similar experiments were performed with histamine and similar results were obtained. From these experiments it was inferred by Heymans and Bouckaert that the leg area during the arterial hypertension resulting from carotid sinus nerve denervation is constricted, whereas the brain vessels take no part in the vaso constriction, in fact are passively stretched, and therefore incapable of further dilation when acetylcholine or histamine are administered.

No criticism may be raised against the formulation that the cerebral blood flow is dependent mainly upon the general arterial pressures but the experiments purporting to show that the cerebral blood vessels are passive are not convincing. Great elevations in systemic arterial blood pressure cause slight and even moderate increases in diameter of cerebral arteries when observed in a properly prepared skull window experiment. However, it is striking that such dilatation is far from maximal, is usually of short duration and is followed by constriction while the blood pressure remains at a constant high level, and never of the degree caused by histamine. If, however, there is even slight mechanical trauma, poor arterialization of the blood, ether, or other anesthesia in excess, cerebral vasodilatation may be so great that histamine causes no perceptible further response. One may not conclude from the experiments of Heymans and Bouckaert that cerebral arteries do not possess independent vasomotor properties.

The purpose of vasomotor function. It is relevant to consider the biological significance of cerebral vasomotor phenomena. The systemic arterial blood pressure is the most important factor in determining the amount of blood flowing through the cerebrum (L. Hill, 1896). Pressor reflexes are exceedingly prompt and effective (Heymans, Bouckaert and Regniers, 1933) in the extracranial regulation of blood pressure in favor of the brain. However, it must be emphasized that the brain is not entirely without intrinsic vasomotor mechanisms, nor is it unprotected against major alterations in blood pressure. The constrictor vasomotor apparatus of the brain probably acts primarily to keep the cerebral vessels in the proper state of constriction and to aid them in

regaining their normal caliber after vasodilatation.[2] Also, the vasoconstrictor apparatus acts as a fine adjustor, regulating blood flow within the head more precisely than the powerful hydrostatic (Bouckaert, 1935, see footnote) or chemical forces (Forbes, Finley, and Nason, 1933). This function of fine adjustment rather than obliterative constriction would appear to be the chief purpose of cerebral vasoconstriction (Forbes, Finley and Nason, 1933). The degree of vasoconstriction may vary from place to place within the cerebrum. Indeed, there appears to be no purpose for obliterative vasoconstriction within the brain, and as a matter of fact, it has never been observed following sympathetic stimulation and probably cannot occur by this means. Actual determination of the magnitude of the vasoconstriction response within the cranium indicates that it is far less than that observed in other parts of the body (skin, Pool, Nason and Forbes, 1934).

Though there are mechanisms for protection against excessive cerebral blood flow, accessory devices for augmenting the flow when the systemic blood pressure happens to be lowered are far more important. If it be assumed that there are no such devices, a dilemma presents itself. For, if during such lowered pressure, the cerebral vessels passively narrowed, then their walls, in collapsing would further reduce the blood flow and allow even less blood to reached the brain tissue. On the other hand, with active dilatation, a fall in blood pressure resulting from a depressor reflex, for example, would be associated with relaxation of cerebral arteries and arterioles and such widening would reduce the peripheral resistance and aid in maintaining adequate cerebral blood flow.

[2] In the personal communication of Bouckaert previously mentioned are cited experiments which dramatically demonstrate the effect of raising the blood pressure with the vessels in a state of active cerebral constriction, as compared to similar elevations when no particular cerebral vasoconstrictor influence is being exerted. Thus, in one experiment a hypertension of a given degree produced only a third as much increase in brain volume if the cerebral vessels had been previously constricted in epinephrine (Bouckaert and Jourdan, 1935).

As a matter of fact, the most recent experiments of Bouckaert and Jourdan (1936), according to these authors, demonstrate these properties. Dogs, in order to eliminate systemic hydrostatic effects, were sympathectomized, leaving only the sympathetic supply to the head intact. Thus, practically all reflex pressor responses to carotid sinus stimulation either endosinusal or electrical, were abolished. With carotid artery hypotension there were changes in cerebral volume and venous pressure which were interpreted as resulting from a slight cerebral vasoconstriction. Inversely, electrical stimulation of the carotid sinus nerve was followed by a slight cerebral vasodilatation.

That there is in fact a mechanism for active cerebral vasodilatation has been demonstrated. For, whether the receptor zone be on the aorta, the bifurcation of the common carotid, or elsewhere, stimulation of depressor afferents, which lowers the blood pressure, dilates the cerebral arterioles.

The aortic and carotid afferents, by preventing excessive blood pressure, conserve cardiac energy. Through those nerves arterial hypertension produces peripheral vasodilatation while hypotension produces peripheral vasoconstriction. Now, it is regularly observed that the pial vasodilatation on stimulation of these afferents starts late,—beginning 15 to 90 seconds after onset of stimulation and usually when the systemic depressor effect has begun, or the fall in blood pressure is well under way. However, the delay in onset of the dilator effect should not be taken too seriously, because the time of onset of dilatation represents merely the time when dilatation first becomes apparent in the vessels under direct inspection. Since the blood pressure on central vagus stimulation usually begins to fall almost at once, the cerebral arteries at first would tend to collapse passively. In fact, in some instances, there is, at first, an actual narrowing which is later followed by dilatation. It is therefore quite likely that the cerebral dilatation occurs simultaneously with, or only slightly after, the general vasodilatation. If there be assumed a common mechanism for brain and other vascular beds, it is conceivable that blood vessels of the brain would dilate at the same time as, or shortly after, the major dilator beds of the body have amply responded. Premature cerebral vasodilatation would be physiologically undesirable because of the excessive cerebral blood flow that would follow, but later dilatation when the blood pressure is descending would advantageously serve the brain while the heart is being spared.

Cerebral vasodilatation may, possibly, also occur with increased cerebral function but as yet there is no demonstration that such vasodilatation is neurogenic. Though delicate neurogenic regulation is conceivable, it is not essential to explain in this fashion such vasodilatation as might accompany augmented function. Carbon dioxide, even in the minute quantities that might accumulate during increased neurone action, is a prompt and effective cerebral vasodilator acting directly on the walls of the minute blood vessels and not necessarily through the intermediation of vasodilator nerves. The vasodilatation of cerebral anemia, asphyxia, and increased intracranial pressure, in all likelihood, are also brought about by carbon dioxide.

Hence, apparently the prime function of neurogenic cerebral vasodilatation is that of compensation, reducing the peripheral resistance of the cerebral vascular tree during periods of depressor reflex action.

Cerebral anemia, asphyxia and increased intracranial pressure. The effects of sudden and presumably complete cerebral anemia have been adequately reviewed (Hill, 1896; de Buck and de Moor, 1901; Pike, Guthrie and Stewart, 1908; Gildea and Cobb, 1930). It is difficult to draw inferences from the experimental data because it is not always certain that the cerebral anemia is complete and also because histological methods are not adequate to separate slightly abnormal neurones from those that are normal.

Cerebral anemia of twenty seconds obliterates the usual action potentials in the cat's cortex (Simpson and Derbyshire, 1934) and anemia of 2 to 15 minutes in most instances causes irreparable changes in the cerebral cortex and medulla. Using histopathological criteria on animals kept alive some time after the period of anemia (Gomez and Pike, 1909) it is seen that the small pyramidal cells of the cerebral cortex are most susceptible to anemia, 8 minutes of occlusion being sufficient to injure many seriously. The Purkinje cells are next in order of sensitiveness with definite chromatolysis perceptible after 13 minutes of anemia. The retina and the medulla are about equally sensitive to anemia and only slightly less so than the cerebellum. The cells of the spinal ganglia are least susceptible, showing no change after 30 minutes of anemia. The neuroglia cells are also unaffected.

In an analogous way, comparison of the relative susceptibility of hind brain functions reveals that reflex effects such as that of swallowing or from stimulation of the cornea are less resistant to anemia than cardiac, respiratory, or vasomotor effects (Pike, Guthrie and Stewart, 1908). Thus, the pupillary and palpebral reflexes have been revived after complete arrest of 15 to 20 minutes of the circulation in the isolated perfused head of the dog; whereas the respiratory and vasomotor functions have been revived after complete circulatory arrest of 30 minutes (Heymans and Bouckaert, 1935).

The usual sequelae of sudden cerebral anemia produced by increasing the intracranial pressure or by occluding the main arterial pathways to the brain are: loss of consciousness, general muscular spasm, respiratory spasm, slow heart, a rise in blood pressure followed by a fall, acceleration of the heart and death. Acceleration of the heart may precede the initial slowing (McDowall, 1933). Respiratory paralysis usually precedes and causes vasomotor paralysis although the reverse may also

occur. Respiration may at first increase in rate, but soon becomes irregular and stops in 30 seconds or 2 minutes (Pike, Guthrie and Stewart, 1908). Convulsions may occur during or after the occlusion (Gildea and Cobb, 1930). It is the interference with cerebral blood flow, not mechanical pressure, that is responsible for these effects (Hill, 1896). If, in dogs, both common and vertebral arteries are simultaneously ligated, about 40 per cent of the animals die within 24 hours; but in the others, collaterals develop between the muscle branches of the vertebral arteries and the other arteries of the cervical and upper intercostal muscles (Andreyev, 1935[I]).

Furthermore, those dogs which survive such a simultaneous ligation of both common and vertebral arteries, usually suffer a temporary or permanent functional disturbance in their higher nervous activity, observations in keeping with the aforementioned histopathological studies. During the first 10 to 12 days after the operation conditioned responses are absent. When restored, at first the ability to make even the grossest discriminations is defective. Later, in some, there is improvement, but the ability to make fine discriminations and long delayed responses is slow to re-establish itself, or may even be permanently lost (Andreyev, 1935[II]).

When the anemia is slow in onset, respiratory disturbances may be absent. In the initial phase blood pressure is moderately high and the heart beat is slow and forceful. Respiration is deep, slow and labored. During the period of recovery, after anemia and compression, Traube-Hering blood pressure waves and Cheyne-Stokes respiration are common. Subsequently, if the anemia continues, the heart accelerates, respiration becomes periodic, with gasps occurring at long intervals and finally the blood pressure falls to zero.

Nearly all dogs will survive the ligation of most arteries supplying the brain if the ligations are performed gradually, at intervals of from 10 to 14 days (Andreyev, 1935[I]). The development of collateral passages begins immediately and is completed in approximately 4 to 6 weeks, although enlargement of the anastomoses may continue for a year or more.

Direct observation of pial arteries reveals that when both common carotid arteries are simultaneously occluded, the flow in the pial arteries promptly slows, though at the same time the arteries increase in diameter (Wolff). The systemic arterial pressure falls to about half its original height (Wolff; Anrep and Starling, 1925). If within half a minute or before the collateral flow is fully established, the common

carotids are released, the pial arteries dilate still further as the circulation speeds up. Dilatation is often so extreme that diapedesis occurs (by skull window observations, Wolff).

Similar effects are also observed when cerebral anemia is produced by distending the lungs with air and thus raising the intrathoracic pressure by 10 to 15 mm. Hg. Stimulation of the common carotids (sinus, nerves, etc.) through operative procedures is avoided by this means. The circulation in the pial vessels slows and the arteries, after brief and slight narrowing, dilate. The systemic arterial pressure falls sharply as the intrathoracic and intracranial venous pressures mount. When the intrathoracic pressure is permitted to return to normal the pial artery diameter becomes still greater (by skull window observations, Wolff and Lennox, 1928).

The effects of cerebral anemia or asphyxia resulting from cerebral compression have been studied intensively (Cushing, 1901, 1902, 1902; Eyster, Burrows and Essick, 1909; Wolff and Forbes, 1928). If the intracranial pressure is raised gradually to the level of the systemic blood pressure, the latter rises sufficiently to exceed the intracranial pressure. Under these circumstances respiration does not change appreciably. If, on the other hand, the intracranial pressure is raised suddenly to the level of the systemic blood pressure, respiration may stop abruptly. The systemic arterial blood pressure then rises steadily, and when it exceeds the pressure of the spinal fluid, respiration begins again. Though rapid, the blood pressure response after cerebral anemia is possibly less prompt than that resulting from the carotid sinus reflexes (Bouckaert and Heymans, 1933). If the intracranial pressure is raised to a level less than the arterial blood pressure (even though as high as 750 mm. H_2O) there is little or no effect on blood pressure, pulse rate, or respiration (Cushing, 1901, 1902, 1902; Eyster, Burrows and Essick, 1909; Wolff and Forbes, 1928). However, when the intracranial pressure is raised to the level of the systemic arterial pressure, there is a prompt blood pressure elevation which is soon followed by a fall. The fall is in turn followed by a rise of the blood pressure to a level above that of the imposed intracranial pressure. Thus, two conflicting forces are set in action by the raised intracranial pressure, namely, peripheral vasoconstriction and a decrease in pulse rate. The decrease in pulse rate at first dominates, as shown by the fall that follows the preliminary rise in blood pressure. The observation that the fall in blood pressure is not as great as would ordinarily occur with a correspondingly slow pulse indicates the effect of vasoconstriction (Eyster, Burrows and Es-

sick, 1909). If the intracranial pressure is raised still further, this series of events repeats itself, and soon the blood pressure assumes a new and higher level, just above the artificially raised intracranial pressure. By this means systemic arterial pressure of 230 mm. Hg or more may be attained. When the intracranial pressure is subsequently lowered to the usual level there is a rapid fall in blood pressure and rise in pulse rate to the pre-existing level (Eyster, Burrows and Essick, 1909).

It was demonstrated by an injection method that the cerebral anemia resulting from increasing the intracranial pressure to a level above the blood pressure is a complete anemia, involving both superficial and deep blood vessels of the brain. If the intracranial pressure is not raised above the systemic arterial blood pressure, the "Circle of Willis" and some of the larger arterial connections at the base of the brain still contain blood. If the intracranial pressure is raised only slightly, the blood vessels at the site of pressure increase are emptied, although the remainder of the brain is seemingly normally supplied (Eyster, Burrows and Essick, 1909).

The intracranial circulation time (time for radium emanation to travel from the internal carotid artery to the internal jugular vein, Wolff and Blumgart, 1929) is about three seconds when the intracranial pressure and systemic arterial blood pressure are at the usual level. The velocity of flow is fundamentally related to the systemic blood pressure. When the systemic arterial blood pressure is elevated, the speed of flow of the blood through the brain is increased; when the systemic arterial pressure is lowered, the speed of flow is decreased, assuming always that the intracranial arterio-venous pressure difference remains constant. Factors that decrease this difference slow the flow; those that increase it increase its speed (Wolff and Blumgart, 1929).

An increase in intracranial pressure at first materially decreases the velocity and is accompanied by a cerebro-vascular dilatation (Wolff and Blumgart, 1929; Wolff and Forbes, 1928). This is followed by a gradual increase in velocity as the systemic arterial blood pressure once more mounts. It has been observed that when, through increasing the intracranial pressure, the arterio-venous pressure difference within the cranium is reduced to approximately one-half the normal, with a corresponding decrease in the velocity of blood flow, the systemic blood pressure rises, and the arterio-venous pressure difference, as well as the velocity of intracranial blood flow, approach their original levels. It has been postulated (Wolff and Forbes, 1928) that this initial cerebrovascular dilatation is a factor in augmenting the volume of intracranial

blood flow during the period of increase in intracranial pressure and before any change in systemic arterial pressure occurs. Whether or not this be so, it is apparent that when the arterio-venous pressure difference becomes sufficiently low, the systemic blood pressure promptly rises. If then, the intracranial pressure is raised to a still greater height, the circulation time within the brain again becomes greatly prolonged. Under conditions of very high intracranial pressure the pial vessels may actually be compressed and emptied of their contents and, unless the arterial blood pressure is once more able to rise above the intracranial venous pressure, the circulation fails and the animal dies (Wolff and Blumgart, 1929). Sudden release of intracranial pressure, which has been high enough to cause cerebral anemia, is followed by prompt dilatation of pial vessels (Cushing, 1901; Wolff and Forbes, 1928) which suggests the reactive hyperemia that has been observed in other tissues under asphyxial conditions (Lewis and Grant, 1925).

Vagus (Cushing 1901) or carotid sinus reflexes (Guernsey, Weisman and Scott, 1933) are in no way essential to the pressor response to increased intracranial pressure since, in the absence of sinus and depressor nerves, the blood pressure is greater, if anything, than the corresponding effect before the nerves are resected. Similarly, the slow pulse of increased intracranial pressure is not dependent on the presence of the carotid sinus nerves (Heymans, Bouckaert and Regniers, 1933).

Cerebral circulation and carbon dioxide. Circulatory adjustments following an increase in the carbon dioxide content of the body occur in favor of the head (by thermocouple in the common carotid and femoral arteries, Bronk and Gesell, 1927). Carbon dioxide is the most important humoral regulator of the cerebral blood flow and certainly the most significant physiological influence upon cerebral artery diameter. An increase in the carbon dioxide content of the arterial blood causes an increase of more than 40 per cent in the cerebral blood flow [as determined by cerebral A-V gas difference, (Lennox and Gibbs, 1932, and Irving and Welch, 1935); as determined by a thermocouple in the internal jugular vein in man (Gibbs, Gibbs and Lennox, 1935).] Cerebral vasodilatation of a corresponding magnitude occurs (Wolff and Lennox, 1930). On the other hand, a decrease in the carbon dioxide results in a decrease in the cerebral blood flow and a moderate decrease in diameter of the pial arteries.

The effects of oxygen are less dramatic. An increase in the oxygen content of the blood causes a slight decrease in cerebral flow and a corresponding decrease in diameter of the arteries. With oxygen lack the

threshold of sensitivity is usually higher and, though there is vasodilatation and increase in flow, it is never of a degree corresponding to that brought about by carbon dioxide. Thus, a well marked anoxemia causes moderate dilatation and an increase in cerebral blood flow. When the degree of oxygen lack is not great, slight decrease in the carbon dioxide tension of the blood may over-shadow the dilatation and blood flow effects of the low oxygen. But if the anoxemia is considerable, then its dilator effect may neutralize the constricting effect of low carbon dioxide. By the same token, anoxemia augments the dilator effect and blood flow effect of increased carbon dioxide, and increased oxygen augments the constrictor and blood flow effects of decreased carbon dioxide. A variation in ratio of the carbonic acid to the bicarbonate of the blood resulting in a relative acidosis causes dilatation and increased flow; the opposite, i.e., a relative alkalosis, causes constriction and decreased flow (Wolff and Lennox, 1930; Lennox and Gibbs, 1932; Gibbs, Gibbs and Lennox, 1935). Actual pH changes were, however, uncertain in their effects (using a thermocouple in the brain substance, Schmidt and Pierson, 1934).

The cerebral blood flow and cerebral artery diameter are altered by changes in blood carbon dioxide amounting to less than one volume per cent (Schmidt and Pierson, 1934). In other words, the threshold of sensitivity of the brain vessels is very low, vasodilatation occurring at a level only slightly above that necessary for respiratory effects and probably less than that necessary for systemic vasomotor effects.

Increase in the diameter of pial arteries and cerebral blood flow with increase of carbon dioxide, although augmented by elevations in blood pressure, are not dependent upon these hydrostatic effects. If the blood pressure is kept constant with a blood pressure compensator, or the systemic arterial pressure is lowered to a level actually less by 15 mm. Hg than that preceding the inhalation of carbon dioxide, cerebral flow is nevertheless augmented (using a thermocouple in the brain substance, Wolff and Cattell, 1935).

In brief, all cerebral vessels, whether medullary, diencephalic or cortical, respond by dilatation to increase in the arterial carbon dioxide. In their reactions to carbon dioxide the cerebral vessels are sensitive, as well as prompt, and the magnitude of their response is great.

The striking effects of carbon dioxide on the cerebral blood vessels are illustrated by the comparative study of the action of carbon dioxide on the vasculature of the leg and of the cerebrum. Thus, decreased ventilation leading to the accumulation of carbon dioxide in the blood

and tissues increases the carotid flow of blood and decreases the femoral flow. The flow through the brain is accelerated in some instances to more than four times the normal rate, whereas the flow through the leg is diminished but not to the same degree of change as in the brain (by comparison of cerebral A-V oxygen difference with that of the leg, Irving and Welch, 1935). In contrast, increased ventilation produces the opposite effects, and none of the changes described are dependent on changes in blood pressure. The augmentation is greatest in the internal carotid, next greatest in the common carotid and absent in the external carotid. These experiments performed on dogs under conditions of artificial respiration demonstrate the reciprocal relation between the carotid and femoral blood flow and the fact, as mentioned above, that circulatory adjustments following an increase in carbon dioxide favor the brain (Bronk and Gesell, 1927).

The above observations are supported by measurements made of the speed of blood flow through the brain and leg of man, by ascertaining the difference in the oxygen content of blood entering and leaving the brain and leg. The degree of circulatory response to changes in blood oxygen and carbon dioxide is greater in the brain than in the leg. Also, blood flow from time to time, and from subject to subject, is more constant in the cerebrum (Lennox and Gibbs, 1932). Also, as in the above, the effects on the circulation of the leg resulting from altering the carbon dioxide content of the blood are opposite to those of the brain. With a normal or excessive arterial oxygen tension, an increased carbon dioxide tension is associated with a decrease in blood flow in the leg. This differential action is apparent only with increased carbon dioxide, and normal or increased oxygen. In fact, with anoxemia, uncertain and parallel effects are often observed. For example, with profound arterial anoxemia, the carbon dioxide tension remaining unchanged, there is markedly increased blood flow in the leg as in the brain. If there be low carbon dioxide with the anoxemia, both brain and leg flow may actually decrease together (by cerebral A-V gas difference Lennox and Gibbs, 1932). The device of a circulatory adjustment physiologically opposite in brain and skeletal muscle is a considerable asset, since such a mechanism permits of an increase in cerebral flow without a change in the systemic arterial pressure.

The rôle of carbon dioxide in giving dominance to the cerebral circulation is further suggested by the effects of rebreathing on the circulation within skeletal muscle (by a thermocouple on the femoral artery, Rein, 1931). In an evaluation of factors that control muscle blood flow,

the action of carbon dioxide on the cerebrum stands in the first rank of importance. The vasoconstriction within muscle that results from re-breathing takes precedence over all other phenomena. Even the increase in blood supply due to work is overcome. After denervation of the leg this vasoconstrictor effect is absent. Close in importance, however, stands the aforementioned vasodilatation associated with increased work, the result of the action of the so-called nutrition regulation mechanisms which dominate all other factors. The vasomotor phenomena concerned with temperature regulation are in the same category. In the third rank stand vasoconstrictor effects released through the carotid sinus reflexes. In animals doing work, sinus pressor reflexes have no effect in decreasing the augmented blood supply of the muscles, whereas carbon dioxide, as mentioned above, not only promptly decreases the blood supply of the muscle but actually reduces the work done (Rein, 1931).

In brief, the dominance of carbon dioxide effects in the innervated muscle contrasts sharply with the effect of carbon dioxide on the intracranial vessels. Carbon dioxide causes the cerebral vessels to dilate in the face of the action of any or all constrictor actions, whereas in properly innervated muscles carbon dioxide causes constriction in the face of most opposing physiological dilator forces.

This subject cannot be dismissed without calling attention to the fact that the re-breathing experiments of Rein are special in a sense, since the amount of carbon dioxide reaching the cerebral vasomotor apparatus is relatively greater than would usually occur when the carbon dioxide is fed into the blood stream from exercising muscle. Commonly carbon dioxide accumulates first in the exercising muscle and then gradually, as the local carbon dioxide mounts, more and more is fed into the blood stream. Seldom, if ever, therefore, would the central carbon dioxide constrictor effect dominate the local dilator effect of carbon dioxide in muscle. However, for purposes of demonstration the special circumstances are allowable.

How carbon dioxide increases the cerebral flow and the diameter of the cerebral vessels is of basic interest. The vasomotor responses are the result of direct hindbrain as well as carotid sinus activity (McDowall, 1935; Heymans, Bouckaert and Regniers, 1933). By sacrificing local needs through constriction of their extensive vascular beds, muscle, mesentery and kidney increase the cerebral flow during increase of carbon dioxide (Ganter, 1926) or cerebral asphyxia (Zuntz, 1878; Rein, 1931; Cushing, 1901; Eyster, Burrows and Essick, 1909). This reduc-

tion in the total vascular bed may, by relatively augmenting blood volume, increase the pressure, or by merely shifting volume, leave blood pressure unaltered. Increasing the cardiac output may be a further factor in raising blood pressure (Grollman, 1930).

But, quite apart from these cardiovascular effects, an increase in carbon dioxide content of the inspired air increases the cerebral blood flow through the aforementioned cerebral vasodilatation. Such increase in flow, as determined by a thermocouple in the parietal cortex, persists in decerebrate animals, in animals with spinal transection, in animals in which the sixth, seventh and eighth cranial nerves have been transected bilaterally, and after the removal of the carotid sinus and vagus nerves and the cervical sympathetic ganglia (Wolff and Cattell, 1935).

These operations could be expected to break most reflex pathways to the cerebral vessels. Efferent vasodilator impulses (Cobb and Finesinger, 1932; Chorobski and Penfield, 1932) traverse the seventh cranial nerve. A small branch, containing most of the vasodilator fibers, leaves the base of the brain and spreads itself upon the surface of the cerebral arteries near the entrance of the internal carotid artery. Hence, when the seventh cranial nerves which supply the parietal cortex with vasodilator nerves are transected in the hindbrain, and when the brain-stem and its pial arteries are transected at the colliculi, and when the cervical sympathetic ganglia are removed, vasomotor nerve connections of this portion of the cerebrum with the hindbrain are severed, preventing the passage of the vasomotor nerve impulses to the corresponding cerebral vessels. Yet when carbon dioxide is administered to the animal under these conditions the brain vessels dilate as before (Wolff and Cattell, 1935).

Moreover, with cervical sympathetic pathways intact, or even stimulated electrically, carbon dioxide inhalation produces only vasodilatation. Hence, the effect of any conceivable vasoconstrictor impulse which reaches the cerebral vasculature is totally annulled by the overwhelming humoral action of the carbon dioxide. Also, vagus and sympathetic stimulation have no effect on the diameter of brain vessels already responding to carbon dioxide (Wolff; Hertzman and Franke, 1935).

The possibility of local vasodilator reflexes of the Lovén or axone reflex variety still remains (Bayliss, 1923; McDowall, 1935). There is, however, no evidence that carbon dioxide can evoke such reflexes in cerebral, or indeed in any other vessels. Moreover, the fact that isolated strips of carotid artery dilate when carbon dioxide is bubbled

through Ringer solution in which such strips are suspended (Cow, 1911) indicates that the vasodilator response to carbon dioxide, in all probability, is not dependent on local reflex effects.

In brief, the cerebral vasodilatation that follows carbon dioxide administration is not dependent on hydrostatic or nervous phenomena. All evidence indicates that the dilatation may result from direct action of carbon dioxide on the walls of cerebral blood vessels.

Brain vascularity and metabolism. Considerations of brain metabolism are a digression from the main topic of cerebral circulation, but since the whole problem has its roots in, and is defined by the brain needs, no discussion of brain vascularity is adequate without a statement of these needs.

In contrast with earlier concepts, it is now appreciated that the oxygen consumption of the brain is high, and in fact, is estimated to be about 10 per cent of the total oxygen utilized by the body (dogs, by cerebral A-V gas difference, Himwich and Nahum, 1932). The magnitude of the oxygen utilization of the ganglion cell may be appreciated when it is realized that the brain represents about 2 per cent of the body weight (man), and as estimated from their volume, only about 1 per cent of the total weight of the cerebrum consists of nerve cells (G. H. Parker, 1922).

Values for the oxygen consumption of the brain, with the "in vitro" method, expressed in cmm./mgm./hour, i.e., Q_{O_2},[3] are as follows: for minced brain or suspension of brain (wet) in Ringer phosphate buffer solution,

Cat cortex	= 0.21	(Shorr, 1935)
Human cortex	= 0.23	(Wortis, 1935)
Ox cortex	= 0.30	(Dixon and Elliott, 1929)
Sheep cortex	= 0.69	(Dixon and Elliott, 1929)
Rabbit whole brain	= 0.70	(Dixon and Elliott, 1929)
Mouse whole brain	= 1.6+	(Holmes, 1930)
Rat whole brain	= 1.2	(Dickens and Simer, 1930)

for slices in Ringer phosphate buffer solution,

Rat brain	= 1.60	(Loebel, 1925)
Rabbit cortex	= 1.20	(Holmes, 1930)
Rabbit white matter	= 0.30	(Holmes, 1930)

[3] All figures are for Q_{O_2} wet. Values reported for Q_{O_2} dry are here converted to Q_{O_2} wet values by dividing by the factor 5, as obtained from figures for the water content of the brain (Donaldson, 1916).

The values for sliced or minced cortex in glucose are generally higher,

Rat cortex, sliced	= 2.9	(Loebel, 1925)
Rat brain, sliced	= 2.3	(Loebel, 1925)
Rat cortex, sliced	= 2.2	(Warburg, 1927)
Rat cortex, suspension	= 2.4	(Dickens and Ŝimer, 1930)

Rat retina has about the same Q_{O_2} (glucose) as cortex, according to Dickens and Ŝimer, namely 2.24, whereas Warburg gives 6.14 (Warburg, Posener and Wegelein, 1925); peripheral nerve and white matter have about the same value, 0.28 (in Ringer phosphate solution) (Holmes and Gerard, 1929).

Higher values of oxygen consumption have been derived by the "in vivo" method. Thus, in dogs,

Q_{O_2} (cmm./mgm./hour):

7.80	(13 cc./100 gms./minute) (Alexander and Czerna, by measurement of cerebral venous outflow, 1913, corrected by Himwich and Nahum, 1932)
8.40	(14 cc./100 gms./minute) (Himwich and Nahum, 1932)
5.7–6	(9.5–10 cc./100 gms./minute) (Winterstein, 1929, quoting Gayda, 1914, who used a Ludwig stromuhr in the internal carotid artery)

Unfortunately, the amount of blood going to the brain still remains an uncertain quantity, and therefore these "in vivo" values for brain oxygen consumption at best are gross approximations. They are based on the values derived by Jensen (1904), and others who essentially followed his technic. The inferences drawn from Jensen's observations are invalid for two reasons: first, because the free anastomoses between the intra- and extracranial circulation make it impossible to determine just how much of the blood from the internal carotid supplies the brain alone; and secondly, because the amount of brain tissue supplied by the internal carotid is variable, and depends upon the amount of blood entering the "Circle of Willis" through its other main sources (Kramer, 1912). Also, the rate and pressure of injection, under the highly artificial circumstances of Jensen's experiments, add more variables and complications. His calculations, therefore, on that portion of the brain supplied by the internal carotid are worthless.

One is confronted, then, with the large discrepancy between these "in vivo" values for brain oxygen consumption and those obtained from "in vitro" experiments. The "in vitro" oxygen consumption of cortex, which is at least twice that of white matter, is only 1/7th or 1/10th that of the "in vivo" values for brain. Since in certain tissues, such as skeletal muscle, for example, the oxygen consumption values by the

"in vivo" and "in vitro" methods essentially agree, (Richardson, Shorr and Loebel, 1930) two possible explanations for the discrepancy in the figures for brain metabolism present themselves. Either the "in vivo" values are not even approximately correct, or the metabolism of brain tissue is more seriously altered by the slicing and mincing, and by the anoxemia and temperature changes incurred during preparation, than are tissues such as skeletal muscle. No definite conclusions at this time are justified.

Despite this discrepancy, a comparison of "in vitro" values for the relative oxygen consumption of different parts of the nervous system in any given animal derived by a single worker, is useful. Also, it is of interest that, according to some data (Dixon and Elliot, 1929) the oxygen utilization of ox cortex (Q_{O_2} 0.30) is not unlike that of its liver, (Q_{O_2} 0.31 – 0.46) and kidney (Q_{O_2} 0.32). Higher values for the kidney than for the brain cortex have, however, been reported (Warburg, 1927).

The respiratory quotients as derived by the "in vivo" and "in vitro" methods agree, and approximate unity (Loebel, rats 0.86; Irving and Welch, dogs 0.95; Dickens and Ŝimer, rats 0.99; Himwich and Nahum, dogs 1.0; Lennox, 1931, humans 0.95).

It may be inferred from the high R.Q. that carbohydrate is involved in brain metabolism. Studies on humans (Myerson and Halloran, 1931; Lennox, 1931) show that the blood on passing through the brain loses some of its carbohydrate. As a matter of fact, the difference in glucose content in arterial and cerebral venous blood is greater than that between arterial and muscle venous blood. For example, there is a difference of 9 mgm. per 100 cc. (Lennox) for arterial and cerebral venous blood as compared with 5 mgm. for blood leaving the arm, and 4 mgm. for blood leaving the leg. Moreover, experimental data suggest that the increased Q_{O_2} of hyperthyroid brain is associated with carbohydrate oxidation (Cohen, 1935).

The fact that the addition of glucose to the substrate increases the oxygen consumption of nerve tissue "in vitro" further supports the suggestion that nerve tissue metabolism involves carbohydrate. The increase is probably dependent upon the conversion of glucose to lactic acid, since there is no increase in oxygen consumption if this conversion is prevented by fluoride (Holmes, 1930). The brain is apparently dependent on blood sugar for lactic acid precursor, rather than any substance it stores, since under aerobic conditions lactic acid rapidly disappears from chopped brain, whereas the glycogen content does not change (Holmes and Holmes, 1926).

Pitted against this view concerning the method of utilization of glu-

cose, is the observation that the R.Q. of the brain remains unity after pancreatectomy in dogs (Himwich and Nahum, 1932). If one assume that in complete diabetes no glucose is oxidized, the high R.Q. may represent some other metabolic process. It is possible, however, that the brain can oxidize glucose without insulin. In short, it is likely that glucose is utilized by the cerebrum, though uncertain is the mode or extent of the glucose utilization and the dependence of the brain on glucose for carbohydrate metabolism (Holmes, 1932 and 1934).

Since the energy requirements of the brain are large and continuous, the adequacy of the brain circulation must be considered with these needs in view. In the first place, the blood constitutes about 5 per cent of the total weight of the brain (Weil, Zeiss and Cleveland, 1931; Ranke, 1915, quoted by Weilet al., 1931) and the blood flow through the brain in any given animal is fairly constant. The values for 100 grams of brain per minute are offered with the aforementioned reservations concerning measurements of cerebral blood flow and are as follows:

Dog and rabbit	=	125 ccm. (Jensen, 1904, measured with a Hürthle stromuhr in the internal carotid, see Hürthle, 1927).
Dog	=	130 ccm. (Gollwitzer-Meier and Eckardt, 1935, measured by a Rein stromuhr on the internal carotids, the occipital and the vertebral arteries).
Dog	=	75 ccm. (Schneider and Schneider, 1934, measured by a Rein stromuhr on the internal carotid).
Rabbit	=	60 ccm. (Winterstein, 1935, by a Fleisch stromuhr in the internal carotid).

From these questionable values the estimated flow for a human brain would be about one liter per minute (Schneider and Schneider, 1934).

The average intracranial circulation time is approximately three seconds (Wolff and Blumgart, 1929). With the possible exceptions of the retina (1.7 seconds) and kidneys (6–8 seconds), the brain circulation time probably does not differ essentially from that of other organs (2.5–5 seconds) (G. N. Stewart, 1921). As mentioned above, increase in the height of the systemic arterial blood pressure is generally associated with an increased cerebral blood flow. Thus, with a blood pressure of 180 mm. Hg (normal 117 mm. Hg) the circulation time is about half that of normal while with the blood pressure of 66 mm. Hg the circulation time is prolonged by 50 per cent (Wolff and Blumgart, 1929).

The highest integrative functions are dependent upon a relatively large and constant flow. Thus, the residual oxygen saturation of the blood in the internal jugular vein after passage through the brain, in

conscious, alert humans is about 60 per cent. But if, on account of a diminished flow it falls to around 35 per cent, mental confusion occurs, and when it falls as low as to approximately 25 per cent, the subject becomes unconscious (Lennox, Gibbs and Gibbs, 1935).

It appears, then, that the amount of blood is large and the flow through the brain rapid. The distribution of the blood within the neuraxis in terms of metabolic activity is clearly demonstrated. The gray matter of the central nervous system is much more richly supplied with capillaries than is the white matter. In fact, in regions studied, the poorest part of the gray is nearly twice as rich in capillary supply as the richest part of the white (Craigie, 1920). Thus, the gray matter of the human cerebral cortex has about 1080 (1000 to 1100) mm. of capillary per cubic millimeter of brain tissue whereas the white matter approximates 300 and seldom more than half the gray (Cobb and Talbot, 1927; Pfeifer, 1933). For purposes of comparison there may be considered the values for cardiac muscle (Wearn, 1924–25) with 11,000 mm. per cubic millimeter of tissue, active skeletal muscle with 6,000 and resting muscle (Krogh, 1924) with 2,000 mm.

Also, regions with rich neurone content are more vascular than those less thickly populated with similar cell forms. Thus, the six-layered neo-cortex is sharply separated from the three-layered paleo-cortex by an abrupt change in capillary pattern, corresponding closely to the alterations in cellular arrangements (Cobb, 1929).

This correlation of capillarity and cell density is demonstrated also in the contrasting capillarity of the different layers of the paleo-cortex itself (Ammon's horn of the rabbit, Pfeifer, 1933; Craigie, 1930, 1931, 1932). In the lamina pyramidalis, a region rich in cells, the average capillary length per cubic millimeter is 680 mm.; in the dentate gyrus, also relatively rich in cellular structures, the capillary count is 400 mm. per cubic millimeter, whereas in the stratum radialis, a fibrous layer, the average is only 300 mm. of capillary per cubic millimeter of tissue (Cobb, 1929).

Furthermore, in the brain-stem, not only is the gray matter twice as rich in capillaries as the white matter but different portions of the gray matter have very distinct differences in vascularity. Thus, the gray correlative centers are in general richer in blood supply than the motor nuclei. For example, the capillarity of the trigeminal motor nucleus is to that of the sensory trigeminal nucleus as 5.3 is to 8.0; the ventral horn of the cord is to the dorsal horn as 6.4 is to 7.2; the molecular layer of the cerebellar cortex is to the granular layer as 7.1 is to 8.7;

the facial motor nucleus is to the dorsal cochlear nucleus as 5.2 is to 10.5 (Craigie, 1920).

There are indications that the oxidation rate and vascularity may also be related. Thus, as mentioned above, the gray matter of the human cerebral cortex has about 1100 mm. of capillary per cubic millimeter of brain tissue, whereas the white matter approximates 300 mm. and seldom more than half the amount of gray. This is comparable to the oxygen consumption in the cortex as compared to the white matter. The rabbit cortex takes up about 1.2 cmm./mgm./hour of oxygen (Holmes, 1932), whereas white matter takes up about 0.2 to 0.3 cmm./mgm./hour, an amount well under half the consumption of the cortex.

Since then, those regions of greatest cell population within the neuraxis have both the greatest vascularity and the greatest oxygen consumption, and since the correlative regions, i.e., those cell collections in which the greatest number of connections are being made, are more vascular than others, it is interesting to consider in which part of the cell unit greatest metabolic activity takes place.

It is to be recalled that a good part of the metabolic activity of the neurone is centered in the ganglion, dendrites and synapses rather than in the peripheral fibers. Since the neurone colony of the spinal ganglion is unique in being without synapses, it has been suggested that should there be an appreciable difference between the metabolism of this cell collection as compared to a similar group with synapses, the difference in metabolism would represent the energy component introduced by synapses (Holmes, 1932).

Several lines of evidence support the view that metabolism in the dorsal root ganglion is low. First, oxygen consumption and glycolysis are no greater than that of peripheral nerve (Holmes, 1932). Secondly, it is common experience that dorsal root ganglia permit of transplantation more readily than do most neurone structures with the exception of peripheral nerves (Cajal, 1928). This indicates that the blood demands are not great. In fact, the spinal root ganglia have been proved histologically to withstand anemia better than do other structures of the neuraxis (Gomez and Pike, 1909).

Furthermore, by actual measurement, the vascularity of the dorsal root ganglion of the cat is revealed to be meager. Quantitative comparison with other nerve tissues indicates the following ratio: cerebral white matter 1.0 (377 millimeters of capillary per cubic millimeter of tissue); trigeminal nerve 1.13 (425 mm./mm.^{3}); trigeminal ganglion 1.38 (519 mm./mm.^{3}); cervical sympathetic ganglion 2.07 (780 mm./mm.)^3; parietal cortex of the cerebrum 2.40 (907 mm./mm.^{3}),

(Dunning and Wolff, 1936). This ratio corresponds closely to that of the aforementioned oxygen consumption of these structures (Holmes 1930, 1932). The layer richest in synapses, namely lamina IV, (Poljak, 1927) was found to be the most vascular layer of the parietal cortex, and incidentally the region which has been shown to be the site of spontaneous, continuous electrical disturbances (O'Leary and Bishop, 1936). Quantitative comparison of the nerve cells in this lamina with those in the trigeminal ganglion (Dunning and Wolff, 1936) revealed that although the number of nerve cells in lamina IV of the cortex was four times greater per unit area than that in the trigeminal ganglion, the cross section area of the nerve cells in any given section of the trigeminal ganglion was twice as great as that of lamina IV of the cortex. This indicates that there is approximately twice the amount of nerve cell tissue in a section of the trigeminal ganglion. Calculated in terms of the cross sectional area of nerve cells, the vascularity of lamina IV of the parietal cortex, the region richest in synapses, is 3.5 times greater than that of the trigeminal ganglion, a structure lacking synapses.

However, even if the metabolism of the spinal ganglion neurone is accepted as being low, it is still uncertain whether this metabolism is necessarily the expression of the lack of synapses, and the greater metabolism of the cortex is the expression of synaptic activity alone. It is conceivable that the difference in metabolism represents merely an innate difference in the metabolism of spinal ganglion neurones as compared to those of the medulla and cortex, especially since it is known that the neurones in the dorsal root ganglia have a less active rôle and are, in fact, actually not essential to the transmission of dorsal root impulses (Bethe, 1897; Steinach, 1899). Thus, though the inference that the synapse is the site of greatest metabolic activity does not follow with certainty, the suggestion is worthy of further experimental consideration.

Now, whether transient increases in function in cerebral ganglia are associated with like increases in brain circulation remains to be considered. If the matter were as simple as in skeletal muscle one could anticipate cerebral vasodilatation and increased flow during greater cerebral activity, and vice versa, vasoconstriction and decreased flow during less activity or sleep. Unfortunately the problem appears to be more complicated. For example, observations on sleep, presumably a period of low cerebral activity, do not show a decrease in cerebral blood flow. Indeed, the slight increase in cerebrospinal fluid pressure during sleep has even been thought to indicate cerebral vasodilatation (Mosso,

1881; Stevenson, Christensen and Wortis, 1929), but actual measurement of brain blood flow does not reveal any appreciable change (as measured by a thermocouple in the internal jugular vein in man, Gibbs, Gibbs and Lennox, 1935).

However, there is some evidence that increase in brain function is followed by an increase in cerebral blood flow. Local brain vasodilatation during periods of increased cerebral function has been experimentally observed. The total capillarity in various portions of the brain has been measured and compared, and it has been observed that an olfactory stimulant held before the nose of a rabbit just before death causes an increase in capillarity in the olfactory lobe, which is not present in either motor or visual cortex. This suggests a reciprocal relationship between increased function and vascularity (Cobb and Talbot, 1927). The inferences from these experiments, however, may be criticised because the stimulant used, ammonium hydroxide, is actually not an olfactory stimulant, and the dilatation in the olfactory lobe may be part of a more generalized reaction to a chemical stimulant in the region of the nasal mucous membranes and the olfactory lobe. The authors dealt with this objection in part by showing that a group of control animals who inhaled another irritant, ether, before death, did not have the increase in capillarity that occurs after ammonia. Hence, though not conclusive, these interesting experiments may not be waived.

By means of the Barcroft gas analysis method, the difference in content of oxygen and carbon dioxide between arterial and cerebral venous blood before and during optical stimulation was measured (Alexander, 1912). It was found that during optical stimulation the difference was reduced. To demonstrate that this was associated with an increased cerebral blood flow, a plethysmograph record of the brain volume was made. It was observed that when the retina was stimulated there was an increase in the volume of the brain, although the blood pressure was maintained constant throughout. It was inferred that increased activity of the brain is associated with increased volume flow of the cerebral blood stream.

Likewise, a light flashed into the eyes of a cat caused a local rise in temperature as measured by a needle thermocouple placed in the optic pathways and optic cortex (Gerard and Serota, 1936). Similarly, pinching, stroking or massaging the feet lead to local cerebral thermal changes as evidenced when the thermocouple was placed in the cutaneous radiations. These changes were interpreted as resulting chiefly from vasodilatation, and were supposedly sequelae of increased function.

"Mental arithmetic," a type of cortical activity involving a minimum of motor acts, was used as a test method for increasing cerebral function. Samples of blood entering and leaving the intracranial cavity before and during the mental performance were compared. During the increased mental function the blood leaving the brain was richer in oxygen and poorer in carbon dioxide as compared with venous blood taken during the control period. These changes were assumed to be due to cerebral vasodilatation during the increased cerebral activity (Lennox and Leonhardt, 1931).

Although further data are necessary to establish the thesis, it appears probable that transient increases in cerebral function are associated with transient increases in cerebral blood supply.

It is therefore reasonable to inquire whether sustained and high grade mentation is accompanied by greater development of the cerebral vascular tree. If such be the case, it is conceivable that the increased development is an acquired response to, or inborn provision for, increased neurone activity. The evidence here is slight and not yet of a statistical nature, though the anatomical data are suggestive. It appears that the cerebral arteries of the brains of intellectuals which have been studied are longer, greater in diameter and have a greater number of branches than those of the average subject (Hindzé, 1926). If we may assume from other anatomical evidence that the number of neurones composing the brain is fixed for the species within certain biological limits (Donaldson, 1932) then, any increase in blood supply would represent an increased demand on the part of more active neurones, rather than the need of a greater cell population. In other words, the better development of the cerebral arterial tree of certain brains may be the accompaniment of sustained exceptional cerebral performance.

Briefly summarized, the situation stands as follows. Oxygen consumption of the brain is large and probably ranks the cerebrum among the more actively metabolizing organs of the body. Since the brain can accumulate no debts, interference with its blood supply is promptly disastrous. It requires and receives a large and constant stream of blood, assured by highly effective local and outlying adjustments. Oxidation and blood supply in some parts of the brain are greater than in others. Those parts in the neuraxis with the largest number of cells and synapses consume the greatest amount of oxygen and also have the greatest vascularity; and even more specifically, those portions of the gray matter where the largest number of correlating connections and synapses occur, receive by far the most abundant supply of blood. Unfortunately, the data on the relation between transient and sustained

increase in cerebral function and brain vascularity are more suggestive than convincing. It is to be hoped that accumulating data from laboratories in various parts of the world will provide further quantitative evidence on this provocative question.

REFERENCES

MUNRO, A. Observations on the structure and functions of the system. Edinburgh, 1783. Quoted from L. H. WEED; Some limitations of the Munro-Kellie hypothesis. Arch. Surg. **18:** 1049, 1929.

KELLIE, G. Edinburgh Medico-Chirurg. Tr. **1:** 84, 1824.

BURROWS, G. On disorders of the cerebral circulation. Lea and Blanchard, Philadelphia, 1848. Longman, Brown, Green and Longmans, London, 1846.

WEED, L. H. The cerebrospinal fluid. Physiol. Rev. **2:** 171, 1922.

WEED, L. H. Some limitations of the Munro-Kellie hypothesis. Arch. Surg. **18:** 1049, 1929.

WEED, L. H. Positional adjustments of the pressure of the cerebrospinal fluid. Physiol. Rev. **13:** 80, 1933.

FLEXNER, L. B. The chemistry and nature of the cerebrospinal fluid. Physiol. Rev. **14:** 161, 1934.

BAYLISS, W. M. AND L. HILL. On intra-cranial pressure and the cerebral circulation. J. Physiol. **18:** 334, 1895.

WEED, L. H. AND L. B. FLEXNER. Further observations upon the Munro-Kellie hypothesis. Bull. Johns Hopkins Hosp. **50:** 196, 1932.

WEED, L. H., L. B. FLEXNER AND J. H. CLARK. The effect of dislocation of cerebrospinal fluid upon its pressure. Am. J. Physiol. **100:** 246, 1932.

FLEXNER, L. B., J. H. CLARK AND L. H. WEED. The elasticity of the dural sac and its contents. Am. J. Physiol. **101:** 292, 1932.

COBB, S. AND D. BLAIN. Arteriosclerosis of the brain and spinal cord. Arteriosclerosis: A survey of the problem. New York, MacMillan Co., 1933. Chapter 14, p. 397.

STRANSKY, E. AND R. LÖWY. Beiträge zur pathologischen Histologie der Hirn- und Meningeal Venenwände. Arbeiten aus dem Neurologischen Institute. Wien. **20:** 331, 1912–13.

COBB, S. XII. The cerebrospinal blood vessels. Cytology and cellular pathology of the nervous system. W. Penfield, Ed. Hoeber, New York, 1932.

BAILEY, P. Peculiarities of the intracranial venous system and their clinical significance. Arch. Neurol. and Psychiat. **32:** 1105, 1934.

STOPFORD, J. S. B. The functional significance of the arrangement of the cerebral and cerebellar veins. J. Anat. **64:** 257, 1930.

O'CONNELL, J. E. A. Some observations on the cerebral veins. Brain **57:** 484, 1934.

COBB, S. Cerebral circulation. VIII. Quantitative study of the capillaries in the hippocampus. Arch. Surg. **18:** 1200, 1929.

CRAIGIE, E. H. On the relative vascularity of various parts of the central nervous system of the albino rat. J. Comp. Neurol. **31:** 429, 1920.

CRAIGIE, E. H. The vascularity of the cerebral cortex of the albino rat. J. Comp. Neurol. **33**: 193, 1921.

COBB, S. AND J. H. TALBOT. Quantitative study of the cerebral capillaries. Tr. A. Am. Physicians **42**: 255, 1927.

PFEIFER, R. A. Die Angioarchitektonik der Grosshirnrinde. Springer, Berlin, 1928.

COBB, S. The cerebral circulation. XIII. The question of "end-arteries" of the brain and the mechanism of infarction. Arch. Neurol. and Psychiat. **25**: 273, 1931.

CEREBRAL BLOOD VESSELS—PHYSICAL CHARACTERISTICS

FORBES, H. S. The cerebral circulation 1. Observation and measurement of pial vessels. Arch. Neurol. and Psychiat. **19**: 751, 1928.

FLOREY, H. W. Microscopical observations on the circulation of the blood in the cerebral cortex. Brain **48**: 43, 1925.

HERTZMAN, A. B. AND F. E. FRANKE. Studies on the pial vessels of the rabbit. Proc. Am. Physiol. Soc., **113**: 63, 1935.

WOLFF, H. G. Unpublished observations on the pial arteries of the cat, using the Forbes skull window.

FORBES, H. S. AND H. G. WOLFF. Cerebral circulation. III. The vasomotor control of cerebral vessels. Arch. Neurol. and Psychiat. **19**: 1057, 1928.

BURTON-OPITZ, R. AND D. J. EDWARDS. Über die durch Hirndruck verursachte Steigerung des Blutdruckes. Wiener Med. Wchnschr. **66**: 506, 1916.

HOWELL, W. H. The influence of high arterial pressures upon the blood flow through the brain. Am. J. Physiol. **1**: 57, 1898.

WOLFF, H. G. AND H. L. BLUMGART. The cerebral circulation. VI. The effect of normal and of increased intracranial blood flow. Arch. Neurol. and Psychiat. **21**: 795, 1929.

GIBBS, F. A. Personal communication.

WOLFF, H. G. AND MCK. CATTELL. Unpublished observations on blood flow. Experiments in the parietal cortex of the cat, using the Schmidt modification of the Gibbs thermopile.

FINLEY, K. H. AND H. S. FORBES. Cerebral circulation. XXII. Venous pressure; effect on caliber of pial arteries. Arch. Neurol. and Psychiat. **29**: 765, 1933.

BOUCKAERT, J. J. AND C. HEYMANS. On the reflex regulation of the cerebral blood flow and the cerebral vaso-motor tone. J. Physiol. **84**: 367, 1935.

SCHMIDT, C. F. AND J. C. PIERSON. The intrinsic regulation of the blood vessels of the medulla oblongata. Am. J. Physiol. **108**: 241, 1934.

GIBBS, F. A. Thermoelectric blood flow recorder in form of a needle. Proc. Soc. Exper. Biol. and Med. **31**: 141, 1933.

DRUGS

FORBES, H. S. AND H. G. WOLFF. Cerebral circulation. III. The vasomotor control of cerebral vessels. Arch. Neurol. and Psychiat. **19**: 1057, 1928.

FORBES, H. S., K. H. FINLEY AND G. I. NASON. Cerebral circulation. XXIV. Action of epinephrine on pial vessels; action of pituitary and pitressin on pial vessels; vasomotor response in pia and in skin. Arch. Neurol. and Psychiat. **30**: 957, 1933.

RISER, M. AND R. SOREL. Effects of epinephrine on the circulation. Compt. rend. Soc. de Biol. **104:** 297, 1930.

RISER, M., P. MERIEL AND PLANQUES. Les Spasms Vasculaires en Neurologie. Étude Clinique et Experimentalé. L'Encephale **26:** 501, 1931.

SCHMIDT, C. F. The intrinsic regulation of the circulation in the hypothalamus of the cat. Am. J. Physiol. **110:** 137, 1934.

WOLFF, H. G. AND McK. CATTELL. Unpublished observations, 1935.

FINESINGER, J. E. AND T. J. PUTNAM. Cerebral circulation. XXIII. Induced variations in volume flow through the brain perfused at constant pressure. Arch. Neurol. and Psychiat. **30:** 775, 1933.

WOLFF, H. G. The cerebral circulation. XIa. The action of acetylcholine. XIb. The action of the posterior lobe of the pituitary gland. XIc. The action of amyl nitrite. Arch. Neurol. and Psychiat. **22:** 686, 1929.

SCHNEIDER, M. AND D. SCHNEIDER. Untersuchungen über die Regulierung der Gehirndurchblutung. II. Mitteilung. Einwirkung verschiedener Pharmaca auf die Gehirndurchblutung. Arch. f. Exper. Pathol. **175:** 640, 1934.

FORBES, H. S., H. G. WOLFF AND S. COBB. The cerebral circulation. X. The action of histamine. Am. J. Physiol. **89:** 266, 1929.

KELLER, C. J. Untersuchungen über die Gehirndurchblutungen. Arch. f. Exper. Pathol. **154:** 357, 1930.

HOWE, H. S. AND E. McKINLEY. Cerebral circulation. Arch. Neurol. and Psychiat. **18:** 81, 1927.

FLOREY, H. W. Microscopical observations on the circulation. Brain **48:** 43, 1925.

LEAKE, C. D., A. S. LOEVENHART AND C. W. MUEHLBERGER. Dilatation of cerebral blood vessels as a factor in headache. J. A. M. A. **88:** 1076, 1927.

FINESINGER, J. E. Cerebral circulation. Effect of caffeine on cerebral vessels. Arch. Neurol. and Psychiat. **28:** 1290, 1932.

WOLFF, H. G. AND H. S. FORBES. The cerebral circulation. IV. The action of hypertonic solutions. Arch. Neurol. and Psychiat. **20:** 73, 1928.

KUBIE, L. S. AND D. M. HETLER. Action of hypertonic solutions; study of circulation in the cortex by means of color photography. Arch. Neurol. and Psychiat. **20:** 249, 1928.

FORBES, H. S. AND G. I. NASON. The cerebral circulation. XLI. Vascular responses to (A) hypertonic solutions and (B) withdrawal of cerebrospinal fluid. Arch. Neurol. and Psychiat. **34:** 533, 1935.

WEIL, A., F. R. ZEISS AND D. A. CLEVELAND. The determination of the amount of blood in the central nervous system after injection of hypertonic solutions. Am. J. Physiol. **98:** 363, 1931.

SCHMIDT, C. F. AND J. C. PIERSON. Intrinsic regulation of the blood vessels of the medulla oblongata. Am. J. Physiol. **108:** 241, 1934.

FINESINGER, J. E. AND S. COBB. The cerebral circulation. XXIV. The action of narcotic drugs on the pial vessels. J. Pharmacol. and Exper. Therap. **53:** 1, 1935.

FORBES, H. S. AND C. C. KRUMBHAAR. Cerebral circulation. XXI. Action of hydrogen sulphide. Arch. Neurol. and Psychiat. **29:** 756, 1933.

POOL, J. L. AND G. I. NASON. Cerebral circulation. XXXV. The comparative effect of ergotamine tartrate on the arteries in the pia, dura and skin of cats. Arch. Neurol. and Psychiat. **33**: 276, 1935.

LENNOX, W. G., E. L. GIBBS AND F. A. GIBBS. Effect of ergotamine tartrate on the cerebral circulation of man. J. Pharmacol. and Exper. Therap. **53**: 113, 1935.

FINESINGER, J. E. AND S. COBB. Cerebral circulation. XXVII. Action on the pial arteries of the convulsants caffeine, absynth, camphor and picrotoxin. Arch. Neurol. and Psychiat. **30**: 980, 1933.

GIBBS, F. A., W. G. LENNOX AND E. L. GIBBS. Cerebral blood flow preceding and accompanying epileptic seizures in man. Arch. Neurol. and Psychiat. **32**: 257, 1934.

CEREBRAL CIRCULATION AND RESPIRATION

ROSENTHAL, J. Studien über Athembewegungen. Arch. f. Anat. u. Physiol., S. 456, 1864; S. 191, 1865.

SCHMIDT, C. F. The influence of cerebral blood flow on respiration. I. The respiratory responses to changes in cerebral blood flow. III. The interplay of factors concerned in the regulation of respiration. Am. J. Physiol. **84**: 202, 1928.

HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS. La Sinus Carotidien et la Zone homologue cardio-aortique. G. Doin et Cie., Paris, 1933.

HILL, L. The physiology and pathology of the cerebral circulation. J. and A. Churchill, London, 1896.

SCHMIDT, C. F. Carotid sinus reflexes to the respiratory center. I. Identification. II. Attempts at evaluation. Am. J. Physiol. **102**: 94, 1932.

BOUCKAERT, J. J. AND C. HEYMANS. Carotid sinus reflexes. Influence of central blood pressure and blood supply on respiratory and vasomotor centers. J. Physiol. **79**: 49, 1933.

WOLFF, H. G. AND W. G. LENNOX. Cerebral circulation. XII. The effect on pial vessels of variations in the oxygen and carbon dioxide content of the blood. Arch. Neurol. and Psychiat. **23**: 1097, 1930.

GREELEY, P. O. AND C. E. GREELEY. Cerebral, head and body flow during periodic ventilation. Proc. Soc. Exper. Biol. and Med. **26**: 828, 1928.

GESELL, R. AND D. W. BRONK. The regulation of respiration. V. A continuous thermoelectric method of recording the volume flow of blood. Am. J. Physiol. **79**: 61, 1926.

HALDANE, J. S. Respiration. Yale University Press, New Haven, 1927.

VASOMOTOR PHENOMENA

HÜRTHLE, K. Blutkreislauf im Gehirn. Handb. der Norm. u. Path. Physiol. **10**: 1, 1927. Julius Springer, Berlin, 1927.

FORBES, H. S. AND H. G. WOLFF. Cerebral circulation. III. The vasomotor control of cerebral vessels. Arch. Neurol. and Psychiat. **19**: 1057, 1928.

HASSIN, G. B. The nerve supply of the cerebral blood vessels. Arch. Neurol. and Psychiat. **22**: 375, 1929.

STOEHR, P., JR. Mikroscopische Anatomie des vegetativen Nervensystems. Julius Springer, Berlin, 1928.

PENFIELD, W. Intracerebral vascular nerves. Arch. Neurol. and Psychiat. **27**: 30, 1932.

CHOROBSKI, J. AND W. PENFIELD. Cerebral vasodilator nerves and their pathway from the medulla oblongata. Arch. Neurol. and Psychiat. **28**: 1257, 1932.

CLARK, S. L. Innervation of choroid plexuses and blood vessels within the central nervous system. J. Comp. Neurol. **60**: 21, 1934.

LEVINE, M. AND H. G. WOLFF. Cerebral circulation. Afferent impulses from the blood vessels of the pia. Arch. Neurol. and Psychiat. **28**: 140, 1932.

PICKERING, G. W. Observations on the mechanism of headache produced by histamine. Clinical Science **1**: 77, 1933.

CLARK, D., H. HOUGH AND H. G. WOLFF. Experimental studies on headache: observations on histamine headache. Sensation: its mechanisms and disturbances. Vol. 15. The Waverly Press, Baltimore, 1935.

THOMAS, C. B. Cerebral circulation: constriction of pial vessels in the unanesthetized cat produced by stimulation of the cervical sympathetic chain. Proc. Am. Soc. Clin. Invest. 27th Annual Meeting, May, 1935.

POOL, J. L., H. S. FORBES AND G. I. NASON. Cerebral circulation. XXXII. Effect of stimulation of the sympathetic nerve on the pial vessels in the isolated head. Arch. Neurol. and Psychiat. **32**: 915, 1934.

FINESINGER, J. E. AND T. J. PUTNAM. Cerebral circulation. XXII. Induced variations in volume flow through the brain perfused at constant pressure. Arch. Neurol. and Psychiat. **30**: 775, 1933.

GOLLWITZER-MEIER, K. AND P. ECKARDT. Weitere Untersuchungen über den Nerveneinfluss auf die Hirndurchblutung. Arch. f. Exper. Path. **177**: 501, 1935.

SCHNEIDER, M. AND D. SCHNEIDER. Untersuchungen über die Regulierung der Gehirndurchblutung. Mitteilung 1. Arch. f. Exper. Path. **175**: 606, 1934.

SCHMIDT, C. F. AND J. C. PIERSON. The intrinsic regulation of the blood vessels of the medulla oblongata. Am. J. Physiol. **108**: 241, 1934.

SCHMIDT, C. F. The intrinsic regulation of the circulation in the hypothalamus of the cat. Am. J. Physiol. **110**: 137, 1934.

SCHMIDT, C. F. The intrinsic regulation of the circulation in the parietal cortex of the cat. Proc. Am. Physiol. Soc. 47th Annual Meeting, April, 1935, p. 115. Also, Am. J. Physiol. (in press).

WOLFF, H. G. AND McK. CATTELL. Unpublished observations, 1935.

TALBOT, J. H., H. G. WOLFF AND S. COBB. The cerebral circulation. VII. Changes in cerebral capillary bed following cervical sympathectomy. Arch. Neurol. and Psychiat. **21**: 1102, 1929.

POOL, J. L., G. I. NASON AND H. S. FORBES. Cerebral circulation. XXXIII. The effect of nerve stimulation and various drugs on the vessels of the dura mater. Arch. Neurol. and Psychiat. **32**: 1202, 1934.

BOUCKAERT, J. J. Personal communication.

BOUCKAERT, J. J. AND F. JOURDAN. Effets de l'Adrenaline et de l'Excitation du Sympathique Cervical sur la Circulation Intracranienne Isolée. Existence de Nerfs Vasoconstricteurs Cerebraux et Action de l'Adrenaline sur les Vaisseaux du Cerveau. Communications, Société Belge de Biologie, June 29, 1935.

PUTNAM, T. J. AND E. ASK-UPMARK. The cerebral circulation. XXIX. Microscopic observations on the living choroid plexus and ependyma of the cat. Arch. Neurol. and Psychiat. **32**: 72, 1934.

PENFIELD, W. AND G. STAVRAKY. The response of intracranial blood vessels to electric stimulation of the thalamus and the hypothalamic regions. Proc. Am. Neurol. Assn., June, 1935.

REIN, H. Ueber Besonderheiten der Blutzirkulation in der Arteria carotis. Ztschr. f. Biol. **89**: 307, 1929–30.

Die Thermo Stromuhr—Ibid. **89**: 195, 1929–30.

BOUCKAERT, J. J. AND C. HEYMANS. On the reflex regulation of the cerebral blood flow and the cerebral vasomotor tone. J. Physiol. **84**: 367, 1935.

COBB, S. AND J. E. FINESINGER. The vagal pathway of the vasodilator impulses. Arch. Neurol. and Psychiat. **28**: 1243, 1932.

ASK-UPMARK, E. The carotid sinus and the cerebral circulation. Acta Psychiatrica et Neurologica, Supp. VI. Lund, 1935.

FORBES, H. S. Personal communication, 1935.

SCHNEIDER, M. AND D. SCHNEIDER. Untersuchungen über die Regulierung der Gehirndurchblutung. Mitteilung III. Die Rolle des Carotissinus bei der Regulierung der Gehirndurchblutung. Arch. f. Exper. Path. **176**: 393, 1934.

GIBBS, F. A. Thermoelectric blood flow recorder in form of a needle. Proc. Soc. Exper. Biol. and Med. **31**: 141, 1933.

FORBES, H. S. Personal communication.

COBB, S. AND J. E. FINESINGER. The vagal pathway of the vasodilator impulses. Arch. Neurol. and Psychiat. **28**: 1243, 1932.

FINLEY, K. H. AND H. S. FORBES. Cerebral circulation. XXII. Venous pressure; effect on caliber of pial arteries. Arch. Neurol. and Psychiat. **29**: 765, 1933.

BOUCKAERT, J. J. AND C. HEYMANS. Carotid sinus reflexes. Influence of central blood pressure and blood supply on respiration and vasomotor centers. J. Physiol. **79**: 49, 1933.

BOUCKAERT, J. J. AND JOURDAN, F.: Sinus carotidiens et circulation cerebrale. Preliminary communication, Société Belge de Biologie, Feb. 29, 1936. To be published in Comptes-rendus de la Société de Biologie.

HILL, L. The physiology and pathology of the cerebral circulation. J. and A. Churchill, London, 1896.

HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS. La Sinus Carotidien et la Zone homologue cardio-aortique. G. Doin et Cie, Paris, 1933.

FORBES, H. S., K. H. FINLEY AND G. I. NASON. Cerebral circulation. XXIV. A. Action of epinephrine on pial vessels. B. Action of pituitary and pitressin on pial vessels. C. Vasomotor response in the pia and in the skin. Arch. Neurol. and Psychiat. **30**: 957, 1933.

CEREBRAL ANEMIA

HILL, L. The physiology and pathology of the cerebral circulation. J. and A. Churchill, London, 1896.

DE BUCK, D. AND L. DE MOOR. Lesions des cellules Nerveuses sous l'influence de l'anemie aigue. Le Nevraxe **2**: 2, 1901.

PIKE, F. H., C. C. GUTHRIE AND G. N. STEWART. Studies in resuscitation: IV. The return of function in the central nervous system after temporary cerebral anemia. J. Exper. Med. **10**: 490, 1908.

GILDEA, E. F. AND S. COBB. The effects of anemia on the cerebral cortex of the cat. Arch. Neurol. and Psychiat. **23**: 876, 1930.

SIMPSON, H. N. AND A. J. DERBYSHIRE. Electrical activity of the motor cortex during cerebral anemia. Proc. Am. Physiol. Soc.; Am. J. Physiol. **109**: 99, 1934.

GOMEZ, L. AND F. H. PIKE. The histological changes in nerve cells due to total temporary anemia of the central nervous system. J. Exper. Med. **11**: 257, 1909.

HEYMANS, C. AND J. J. BOUCKAERT. Sur la Survie et la Reviviscence des Centres Nerveux Encephalo-Bulbaires. Summaries of Communications, Int'l Physiol. Cong. Leningrad-Moscow, 1935, p. 156.

MCDOWALL, R. J. S. A chemical control of the heart. Quart. J. Exper. Physiol. **23**: 270, 1933.

ANDREYEV, L. A. Functional changes in the brain of the dog after reduction of cerebral blood supply: I. Cerebral circulation and the development of anastomosis after ligation of the arteries. Arch. Neurol. and Psychiat. **34**: 481, 1935.

ANDREYEV, L. A. Functional changes in the brain of the dog after reduction of cerebral blood supply: II. Disturbances of conditioned reflexes after ligation of arteries. Arch. Neurol. and Psychiat. **34**: 699, 1935.

WOLFF, H. G. Unpublished observations.

ANREP, G. V. AND E. H. STARLING. Central and reflex regulation of the circulation. Proc. Roy. Soc. London, Series B, **97**: 463, 1925.

WOLFF, H. G. AND W. G. LENNOX. Encephalitic respiratory sequelae. A clinical and experimental study. J. Nerv. and Ment. Dis. **68**: 337, 1928.

CUSHING, H. Concerning a definite regulatory mechanism of the vasomotor center which controls blood pressure during cerebral compression. Bull. Johns Hopkins Hosp. **12**: 290, 1901. (No. 126)

CUSHING, H. Physiologische und Anatomische Beobachtungen über den Einfluss von HirnKompression auf den intracraniellen Kreislauf und über einige hiermit verwande Erscheinungen. Mitt. a. d. Grenz. der Med. u. Chir. **9**: 773, 1902.

CUSHING, H. Some experimental and clinical observations concerning states of increased intracranial tension. Am. J. M. Sc. **124**: 376, 1902.

EYSTER, J. A. E., M. T. BURROWS AND C. R. ESSICK. Studies on intracranial pressure. J. Exper. Med. **11**: 489, 1909.

WOLFF, H. G. AND H. S. FORBES. Cerebral circulation. V. Observations on the pial circulation during changes in intracranial pressure. Arch. Neurol. and Psychiat. **20**: 1035, 1928.

BOUCKAERT, J. J. AND C. HEYMANS. Carotid sinus reflexes. Influence of central blood pressure and blood supply on respiratory and vasomotor centers. J. Physiol. **79**: 49, 1933.

WOLFF, H. G. AND H. L. BLUMGART. The cerebral circulation. VI. The effect of normal and increased cerebrospinal fluid pressure on the velocity of intracranial blood flow. Arch. Neurol. and Psychiat. **21**: 795, 1929.

LEWIS, T. AND R. GRANT. Observations upon reactive hyperemia in man. Heart **12**: 73, 1925.

GUERNSEY, C. M., S. A. WEISMAN AND F. H. SCOTT. Effect on the reflexes of the carotid sinus of raising the intracranial pressure. Arch. Int. Med. **52**: 306, 1933.

HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS. La Sinus Carotidien et la Zone homologue cardio-aortique. G. Doin et Cie., Paris, 1933, p. 48.

CARBON DIOXIDE

BRONK, D. W. AND R. GESELL. The regulation of respiration. X. Effects of carbon dioxide, sodium bicarbonate and sodium carbonate on the carotid and femoral flow of blood. Am. J. Physiol. **82**: 170, 1927.

LENNOX, W. G. AND E. L. GIBBS. The blood flow in the brain and the leg of man, and the changes induced by alteration of blood gases. J. Clin. Invest. **11**: 1155, 1932.

IRVING, L. AND M. S. WELCH. The effect of the composition of the inspired air on the circulation through the brain. Quart. J. Exper. Physiol. **25**: 121, 1935.

GIBBS, F. A., E. L. GIBBS AND W. G. LENNOX. Changes in human cerebral blood flow consequent on alterations in blood gases. Am. J. Physiol. **111**: 557, 1935.

WOLFF, H. G. AND W. G. LENNOX. Cerebral circulation. XII. The effect on pial vessels of variations in the oxygen and carbon dioxide content of the blood. Arch. Neurol. and Psychiat. **23**: 1097, 1930.

SCHMIDT, C. F. AND J. C. PIERSON. The intrinsic regulation of the blood vessels of the medulla oblongata. Am. J. Physiol. **108**: 241, 1934.

WOLFF, H. G. AND McK. CATTELL. Unpublished observations, 1935.

REIN, H. Vasomotorische Regulationen. Ergebn. d. Physiol. **32**: 28, 1931.

MCDOWALL, R. J. S. The nervous control of the blood vessels. Physiol. Rev. **15**: 98, 1935.

HEYMANS, C., J. J. BOUCKAERT AND P. REGNIERS. La Sinus Carotidien et la Zone homologue cardio-aortique. G. Doin et Cie. Paris, 1933.

GANTER, G. Über die Wirkung der Asphyxie auf die Arterien und der Kreislauf. Gefassstudien: II. Mitteilung. Arch. f. Exper. Pathol. **112**: 66, 1926.

ZUNTZ, N. Ueber die Wirkungen des Stickoxydulgases. Beitrag zur Kenntniss der Einwirkungen der Athmung auf den Kreislauf. Pflüger's Arch. **17**: 135, 374, 1878.

CUSHING, H. Concerning a definite regulatory mechanism of the vasomotor center which controls blood pressure during cerebral compression. Bull. Johns Hopkins Hosp. **12**: 290, 1901.

EYSTER, J. A. E., M. T. BURROWS AND C. R. ESSICK. Studies on intracranial pressure. J. Exper. Med. **11**: 489, 1909.

GROLLMAN, A. Physiological variations in the cardiac output of man. Am. J. Physiol. **94**: 287, 1930.

COBB, S. AND J. E. FINESINGER. The vagal pathway of the vasodilator impulses. Arch. Neurol. and Psychiat. **28**: 1243, 1932.

CHOROBSKI, J. AND W. PENFIELD. Cerebral vasodilator nerves and their pathway from the medulla oblongata. Arch. Neurol. and Psychiat. **28**: 1257, 1932.

WOLFF, H. G. Unpublished observations.

HERTZMAN, A. B. AND F. E. FRANKE. Studies on the pial vessels of the rabbit. Proc. Am. Physiol. Soc., 1935, p. 63.

BAYLISS, W. M. The vasomotor system. Longmans, Green and Co., London, 1923.

COW, D. Some reactions of surviving arteries. J. Physiol. **42**: 125, 1911.

BRAIN VASCULARITY AND METABOLISM

HIMWICH, H. E. AND L. H. NAHUM. The respiratory quotient of the brain. Am. J. Physiol. **101**: 446, 1932.

PARKER, G. H. The evolution of man. Vol. ii, p. 93. Yale University Press, New Haven, 1922.

DONALDSON, H. H. A revision of the percentage of water in the brain and in the spinal cord of the albino rat. J. Comp. Neurol. **27**: 77, 1916–17.

SHORR, E. Personal communication.

WORTIS, S. Personal communication.

DIXON, M. AND K. A. C. ELLIOTT. XC. The effect of cyanide on the respiration of animal tissues. Biochem. J. **23**: 812, 1929.

HOLMES, E. G. Oxidation in central and peripheral nervous tissues. Biochem. J. **24**: 914, 1930.

DICKENS, F. AND F. ŜIMER. The metabolism of normal and tumor tissue. The R.Q. and the relationship of respiration of glycolysis. Biochem. J. **24**: 1301, 1930.

LOEBEL, R. O. Beiträge zur Atmung und Glykolyse tierische Gewebe. Biochem. Ztschr. **161**: 219, 1925.

WARBURG, O. Über die Klassifierung tierische Gewebe nach ihrem Stoffwechsel. Biochem. Ztschr. **184**: 484, 1927.

WARBURG, O., R. POSENER AND E. WEGELEIN. Über den Stoffwechsel der Carcinomzelle. Biochem. Ztschr. **152**: 309, 1925.

HOLMES, E. G., AND R. W. GERARD. LXXIV. Studies on nerve metabolism. IV. Carbohydrate metabolism of resting mammalian nerve. Biochem. J. **23**: 738, 1929.

ALEXANDER, F. G. AND S. CZERNA. Einfluss der Narkose auf den Gaswechsel des Gehirns. Biochem. Ztschr. **53**: 100, 1913.

WINTERSTEIN, H. Der Stoffwechsel des Zentral Nervensystem. Handb. der Norm. u. Path. Physiol. **10**: 515, 1929.

JENSEN, P. Über die Blutversorgung des Gehirns. Pflüger's Arch. **103**: 171, 1904.

KRAMER, S. On the function of the circle of Willis. J. Exper. Med. **15**: 348, 1912.

RICHARDSON, H. B., E. SHORR AND R. O. LOEBEL. Tissue metabolism. II. The respiratory quotient of normal and diabetic tissue. J. Biochem. **86**: 551, 1930.

IRVING, L. AND M. S. WELCH. The effect of the composition of the inspired air on the circulation through the brain. Quart. J. Exper. Physiol. **25**: 121, 1935.

LENNOX, W. G. The cerebral circulation. XIV. The respiratory quotient of the brain and of the extremities of man. Arch. Neurol. and Psychiat., **26**: 719, 1931.

MYERSON, A. AND R. D. HALLORAN. Studies of biochemistry of brain blood by internal jugular puncture. Bull. Mass. Dept. Ment. Dis. **15**: 9, 1931.

COHEN, R. A. Hyperthyroidism and brain oxidations. Proc. Soc. Exper. Med. and Biol. **32**: 1446, 1935.

HOLMES, E. G. AND B. E. HOLMES. CLII. Contributions to the study of brain metabolism. Carbohydrate metabolism. Relationship of glycogen and lactic acid. Biochem. J. **20**: 1196, 1926.

HOLMES, E. G. The metabolism of brain and nerve. Annual review of biochemistry, Vols. I and III. Stanford University Press, Stanford University, California. 1932 and 1934.

WEIL, A., F. R. ZEISS AND D. A. CLEVELAND. The determination of the amount of blood in the central nervous system after injection of hypertonic solutions. Am. J. Physiol. **98**: 363, 1931.

RANKE, O. Zur Histologie and Histopathologie der Blutgefasswand Specziell der Zentral Nervensystems. Ztschr. f. d. ges. Neur. u. Psych. **27**: 221, 1915.

HÜRTHLE, K. Blutkreislauf im Gehirn. Handb. der Norm. u. Path. Physiol. **10**: 1, 1927.

GOLLWITZER-MEIER, K. AND P. ECKARDT. Weitere Untersuchungen über den Nerveneinfluss auf die Hirndurchblutung. Arch. f. Exper. Pathol. **177**: 501, 1935.

SCHNEIDER, D. AND M. SCHNEIDER. Über die Regulierung der Durchblutungung des Gehirns. Arch. f. Exper. Pathol. **175**: 606, 1934.

WINTERSTEIN, H. Über den Blutkreislauf Kaninchenhirn. Pflüger's Arch. **235**: 377, 1935.

WOLFF, H. G. AND H. L. BLUMGART. The cerebral circulation. VI. Effect of normal and increased intracranial cerebrospinal fluid pressure on the velocity of intracranial blood flow. Arch. Neurol. and Psychiat. **21**: 795, 1929.

STEWART, G. N. Researches on the circulation time and on the influences which affect it. V. The circulation time of the spleen, kidney, intestine, heart (coronary circulation) and retina, with some further observations on the lesser circulation. Am. J. Physiol. **58**: 278, 1921–22.

LENNOX, W. G., F. A. GIBBS AND E. L. GIBBS. Relationship of unconsciousness to cerebral blood flow and to anoxemia. Arch. Neurol. and Psychiat. **34**: 1001, 1935.

CRAIGIE, E. H. On the relative vascularity of various parts of the central nervous system of the albino rat. J. Comp. Neurol. **31**: 429, 1920.

PFEIFER, R. A. Die Angioarchitektonik der Grosshirn. Verhandl. d. deutsch. Gesellsch f. Kreislaufforsch. Springer, Berlin, 1933.

COBB, S. AND J. H. TALBOT. Quantitative study of cerebral capillaries. Trans. Assn. Am. Physicians **42**: 255, 1927.

WEARN, J. T. Studies of the capillaries and Thebesian vessels of human and cat hearts. J. Clin. Invest. **1**: 572, 1924–25.

KROGH, A. The anatomy and physiology of the capillaries. pp. 232–238. Yale University Press, New Haven, 1924.

COBBS, S. Cerebral circulation. VIII. Quantitative study of the capillaries in the hippocampus. Arch. Surg. **18**: 1200, 1929.

CRAIGIE, E. H. The vascular supply of the archicortex of the rat. J. Comp. Neurol. **51**: 1, 1930.

CRAIGIE, E. H. Ibid. **52**: 353, 1931.

CRAIGIE, E. H. Ibid. **55**: 443, 1932.

HOLMES, E. G. The metabolic activity of the cells of the trigeminal ganglion. Biochem. J. **26**: 2005, 1932.

RAMON Y CAJAL, S. Degeneration and regeneration of the nervous system. Oxford Univ. Press, London, 1928.

GOMEZ, L. AND F. H. PIKE. The histological changes in nerve cells due to total temporary anemia of the central nervous system. J. Exper. Med. **11**: 257, 1909.

DUNNING, H. S. AND H. G. WOLFF. The relative vascularity of the trigeminal ganglion and nerve, cerebral cortex and white matter. Trans. Am. Neurol. Assn., 1936.

POLJAK, S. An experimental study of the association callosal, and projection fibers of the cerebral cortex of the cat. J. Comp. Neurol. **44**: 197, 1927.

O'LEARY, J. AND G. H. BISHOP. The source of cortical potentials. Proc. Am. Physiol. Soc. March, 1936, p. 115.

BETHE, A. Das Centralnervensystem von Carcinas Maenas. Ein anatomisch-physiologischer Versuch. I Theil. II Mitteilung. Arch. f. Mikrosk. Anatomie **50**: 589, 1897.

STEINACH, E. Ueber die centripetale Erregungsleitung im Bereiche des Spinalganglions. Pflüger's Arch. **78**: 291, 1899.

MOSSO, A. Ueber den Kreislauf des Blutes im menschlichen Gehirn. Untersuchungen. Von Veit und Comp., 1881.

STEVENSON, L., B. E. CHRISTENSEN AND S. B. WORTIS. Some experiments in intracranial pressure in man during sleep and under certain other conditions. Am. J. Med. Sci. **178**: 663, 1929.

GIBBS, F. A., E. L. GIBBS AND W. G. LENNOX. Cerebral blood flow during sleep in man. Brain **58**: 44, 1935.

GERARD, R. W. Nerve metabolism. Physiol. Rev. **12**: 469, 1932.

ALEXANDER, F. G. Untersuchungen über den Blutgaswechsel des Gehirns. Biochem. Ztschr. **44**: 127, 1912.

GERARD, R. W. AND H. SEROTA. Localized thermal changes in brain. Proc. Am. Physiol. Soc., **116**: 59, 1936.

LENNOX, W. G. AND E. LEONHARDT. The cerebral circulation. XII. The effect of mental work. Arch. Neurol. and Psychiat. **26**: 725, 1931.

HINDZÉ, B. Les Artéres du Cerveau des Hommes d'Elite. Extrait des Bulletins et Mémoires de la Société d'Anthropologie de Paris. (Séance du 2 décembre, 1926.)

DONALDSON, H. H. The brain problem—in relation to weight and form. Am. J. Psychiat. **12**: 197, 1932.

CARDIAC METABOLISM

E. W. H. CRUICKSHANK

Department of Physiology, Marischal College, The University, Aberdeen

THE GLYCOGEN CONTENT OF THE HEART. The average glycogen content of the frog's heart is 0.54 g./100 g.; the total carbohydrate content is 1.42 g./100 g. (Clark, Gaddie and Stewart, 1930, 1931; Wertheimer, 1930). The glycogen content of the mammalian heart varies somewhat as to the species of animal used. The distribution of glycogen in the mammalian heart has been studied by several (Cruickshank and Shrivastava, 1929; Macpherson, Essex and Mann, 1931–32). It is generally highest in the auricles, next highest in the septum (Blume, 1934) with the ventricles containing slightly less. Cardiac glycogen suffers rapid post-mortem destruction (Loeper and Lemaire, 1930). Doubt has been expressed by Yater, Osterberg and Hefke (1928) as to whether or not the Bundle of His contains more glycogen than the ventricular muscle (Pace, 1932). Excised and perfused hearts show great variations in their glycogen content; for example, the glycogen of the cat's heart falls after two hours' perfusion with saline from an average of 0.483 to 0.152 g./100 g. (Macpherson *et al.*, 1931–32; Tanzi, 1933, 1934; Witting, Markowitz and Mann, 1930; Sprague, 1933). Similar losses have been noted by Camis (1934) and Cruickshank and McClure (1936).

The most striking change in the glycogen figure is to be seen in diabetes; invariably there is an increase. In 1913 Cruickshank showed that diabetic hearts may have as much as 45 per cent more glycogen than the normal. This has been confirmed by Fisher and Lackey (1925), Kosterlitz (1933), and by Cruickshank and Startup (1933b) using the improved Pflüger method of Sahyun (1931). The changes in cardiac glycogen due to hypoglycemia are but part of the greater depletion of glycogen seen when the mammalian heart has continued beating for two or three hours under strictly aglycemic conditions. A moderate degree of hypoglycemia (blood sugar = 0.04 g./p.c.) produces an average loss of 20 per cent (Cruickshank and Startup 1933a; Evans, 1934); in aglycemia in which blood sugar has been normally and completely utilized by the heart the loss may not average

more than 35 per cent, i.e., a fall from normal of 0.686 to 0.449 g./100 g. (Cruickshank and McClure, 1936). A detailed investigation of the glycogen content of the heart of rats under various experimental conditions has been carried out by Evans (1934). Important in his findings is the constancy of the values for heart glycogen. Twenty normal hearts contained 0.508 ± 0.012 g./100 g. Extreme values were 0.444 and 0.675 g. Fasting and exercise did not reduce the glycogen; anoxemia, by inspiring air with 12 to 9 per cent of oxygen had no effect. Extreme degrees of anoxemia (O_2 = 7 to 5 per cent) reduced the glycogen some 20 to 60 per cent. With or without exercise, starvation of 24 to 48 hours does not reduce heart glycogen (Kulz, 1891; Macleod and Prendergast, 1921; Lawrence and McCance, 1931). Longer periods of starvation, 3 to 56 days, were equally unsuccessful in reducing cardiac glycogen (Bong, Junkersdorf and Steinborn, 1934).

Insulin in the presence of ample blood sugar produces a marked deposition of cardiac glycogen while in the absence of blood sugar it apparently conserves the carbohydrate stores (Cruickshank and Startup, 1933a, b; Bong *et al.*, 1934; Evans, 1934). Starvation of 24 hours' duration followed by insulin in the whole animal, results in no change in glycogen (Lawrence and McCance, 1931). In diabetes insulin reduces cardiac glycogen toward the normal. From all observations it is true that insulin effects a deposition of glycogen in the heart.

Opinion is divided with regard to the action of adrenaline. That it causes no loss in the glycogen of the heart muscle is the finding of Bong *et al.* (1934). Junkersdorf and Török (1926) stated that epinephrine administered daily to fasting dogs over a period of nine days resulted in a slight increase of heart glycogen: their figures for four experiments, 0.86, 0.57, 0.66, 0.54 are, however, not conclusive. More definite results by Junkersdorf and Hanisch (1927), however, support this conclusion, which receives further confirmation from Geiger and Schmidt (1928). A loss of about 45 per cent of heart glycogen upon daily adrenaline administration to dogs for seven days was reported by Haendel and Munilla (1929), average figures showing a fall from a normal of 0.561 to 0.334 g./100 g. In the isolated heart lung preparation adrenaline does not have the specific effect on heart glycogen which is characteristic for skeletal muscle glycogen. To obtain a marked fall of glycogen, in a heart well supplied with carbohydrate, it is necessary to administer large doses of adrenaline (Bogue *et al.*, 1935).

THE GASEOUS METABOLISM OF THE "COLD BLOODED" HEART. In considering carbohydrate metabolism one must always discriminate

between processes occurring in different types of muscle *per se* and those which occur in mixtures of muscle pulp with saline or serum. Critical considerations demand a knowledge of the carbohydrate content of the heart and perfusing fluid at the beginning and end of the experimental period, a comparison of resting and active metabolism and an estimation of the amount of sugar used and the work done. It is manifest that until recently little attempt has been made to meet such demands.

The cold blooded heart. The early work of Johannes Müller (1904) was without adequate controls; a saline perfusion fluid with a heart concerning whose activity no statement was made showed a definite loss of sugar after seven hours. Locke and Rosenheim's work (1907) showed that the non-beating heart used as much carbohydrate as the active one and that the removal of Ca or K from the perfusing fluid had no effect upon the rate of disappearance of sugar. That one need expect little difference between the sugar utilization of the still and beating cold blooded heart is generally maintained (Eismayer and Quincke, 1929). Wertheimer (1930) has carefully investigated the carbohydrate metabolism of the frog's heart almost month by month and has concluded that the glycogen of the working heart was not diminished, that there was no relation between the duration and the amount of work and the glycogen content of the heart, that there could be no support for the idea that heart glycogen was used entirely for cardiac work, that in aerobic conditions circulating sugar was used and not glycogen while in anaerobic conditions glycogen was utilized. Wertheimer (1933) using strips of frog's heart has also shown that only after a definite weight limit or load has been passed does the muscle show any loss of its carbohydrate, that never within the optimum load limit, which varied month by month, did the heart lose glycogen nor was there any evidence of a relation between carbohydrate used and work done. He also stated that increasing the Ca or K of the perfusing fluid causes an increase in glycogen loss up to 31 and 12 per cent for Ca and K respectively. The figures for the loss of glycogen occasioned by treatment of cardiac strips with various drugs are of no great value. The most important fact arising from this work is that up to a point of maximal efficiency occasioned by increase in initial length of the strip there is apparently no call upon carbohydrates for energy purposes.

This leads to a discussion of the important work of Clark and his colleagues in Edinburgh. They have shown (1931) that when the frog's heart is perfused with Ringer's fluid a certain amount of sugar is excreted by the heart, that when serum with 0.01 per cent sugar is added

no such excretion takes place. This supports an earlier criticism of such perfusion methods, namely, that under abnormal conditions the normal contents of the heart muscle may be washed out in the first hour or two of the perfusion. In a later paper (1932) they showed that hearts suffering from a lack of oxygen rapidly turn carbohydrate into lactic acid thus accounting for a loss in carbohydrate. The most important fact emphasized by Clark *et al.* (1931) is that the isolated heart of the frog beating in air or oxygen derives its energy, at least in part, from sources other than carbohydrate. A further step in their investigations was to discover the conditions under which the heart would utilize carbohydrate in preference to non-carbohydrate material. The perfusion fluid which best facilitated the burning of sugar by the heart was heparinized blood *plus* one unit of insulin per cubic centimeter of fluid. While Clark *et al.* (1931) maintain that the addition of 0.1 cc. glucose to the perfusion fluid causes no increase in oxygen used, Eismayer and Quincke (1930) maintain that it does. Clark *et al.* have found that the amount of carbohydrate disappearing from the heart when it is perfused with sugar-free Ringer's fluid amounts to 25 per cent of total metabolism; when, however, sugar is added to the perfusion fluid (Ringer *plus* serum) the amount of carbohydrate removed from the heart remains unchanged and the carbohydrate removed from the perfusion fluid amounts to about 20 per cent of the total metabolism, that is to say, the addition of sugar to the perfusing fluid does not spare glycogen but raises the carbohydrate metabolism some 45 per cent of the total metabolism. With the use of heparinized blood, however, the amount of carbohydrate removed from the perfusing fluid is practically unaltered but there is a slight increase in the heart's total carbohydrate. While the addition of insulin to Ringer's solution does not lead to a storage of glycogen its addition to perfusing blood causes a very definite synthesis of cardiac glycogen with a maintenance of the rate of utilization of carbohydrate by the heart. The initial difficulty in Clark's experiments arises from the apparent production of a certain amount of reducing substances from some non-reducing source, which requires the use of 6 cc. oxygen per gram of heart over a period of four hours to bring the heart to the level of control values. To balance the oxygen consumption with the metabolic changes they have assumed the production, during perfusion, of reducing and fermentable substances amounting to 20 per cent of the initial content of reducing substances in the heart. The experiments of Clark and his co-workers go to show that the perfused frog's heart

loses from 26 to 29 per cent of its total carbohydrate in six hours and from 37 to 66 per cent in twenty-four hours' perfusion, that a little sugar is excreted in the first six hours, that the average sugar utilization is 0.576 and 0.266 mgm. per gram of heart muscle per hour for six and twenty-four hour perfusions and that the oxygen utilization is 1.51 and 0.85 cc. per gram per hour for six and twenty-four experiments respectively. This means that over 70 per cent of the cardiac metabolism is other than carbohydrate. The following table forms the basis of these calculations:

PERFUSION FLUID—OF EXPERIMENT	TIME	TOTAL REDUCING SUBSTANCE		PER CENT LOSS OF REDUCING SUBSTANCE	SUGAR EXCRETED	SUGAR USED	OXYGEN USED
		Control heart	Perfused heart				
	hours	*mgm./g.*	*mgm./g.*		*mgm./g./hr.*	*mgm./g./hr.*	*cc./g./hr.*
Ringer's (1931)	6	14.2	15.7		0.225		1.26
	24	14.2	7.3	48.5	0.125	0.162	0.75
Ringer-serum (1931)	6	14.2	10.5	26.0		0.616	1.90
	24	14.2	4.8	66.0		0.392	0.95
Ringer's (1932)	6	12.28	8.70	29.0	0.06	0.537	1.38
	24	15.53	9.66	37.8		0.244	
Ringer Ca poor	6	12.28	14.17		0.027		0.43
	6	11.97	13.30		0.166		0.45

Oxygen utilization of the frog's heart and its relation to work. In 1910 Rhode determined the R.Q. of the frog's heart and from the high figures obtained he concluded that the heart used sugar. He found that much more oxygen was used than could account for the sugar lost and he assumed that protein and fat were utilized subsequent to the loss of sugar. The interest this created in oxidation processes in connection with energy metabolism led to the work of Weizsacker (1911) and Bodenheimer (1916) who found that work performed by the heart was independent of oxygen utilization. Bohnencamp and Ernst (1927) from a study of the heat production of the heart concluded that an equal amount of energy was released with each contraction and that this amount of energy was not altered by any changes which may occur in the initial filling of the frog's ventricle. Lüscher (1920–1921) stated that with a frog's ventricle the amount of oxygen used was not

dependent on diastolic volume but with a constant diastolic volume oxygen utilization varied according to factors or mechanical conditions associated with systole. Arterial resistance for example if changed from 10 to 40 mm. Hg increased oxygen consumption about 20 per cent. Bohnencamp, Eismayer and Ernst (1928) found that changes in diastolic volume of the ventricle from zero to the highest compatible with the heart's action were without influence on the oxygen consumption. With the exception of the work of Rhode (1912) researches upon the mammalian heart by several investigators failed to confirm these statements of Lüscher. Clark and White (1928) in their earlier work on the frog's heart state definitely that oxygen consumption due to contraction varies as the diastolic volume of the heart and is not affected by systolic events such as variations in the resistance thus supporting the view that the Fenn effect does not occur in heart muscle. This was further corroborated by Fischer who stated (1927) that an increase in initial tension gives rise to an increase in heat production and an increase in the work done. An important paper in this connection is that by Clark and White (1929) on the oxygen consumption of the frog's auricle. It is pointed out that, because the thickness of the auricular wall in relation to its diameter is so small, the Warburg formula $d = \sqrt{8c.\ D/A}$, (where d = thickness in centimeter, A = observed oxygen consumption in cc./g./minute, c = concentration of oxygen in atmospheres, D = Krogh's diffusion co-efficient for oxygen which is 1.4×10^{-5} for muscle at 20°C.), can be applied in determining the thickness of the tissue. By a comparison of calculated and estimated thicknesses it was found that the filled and empty auricle is adequately supplied with oxygen if it be suspended in an atmosphere of oxygen; if it be suspended in air the filled auricle will receive by diffusion just enough oxygen but the empty auricle will not. The auricle was filled with a mixture of equal parts of serum and Ringer's fluid of the percentage composition, NaCl 0.65, $CaCl_2$ 0.024, KCl 0.015 and sodium phosphate 0.02 (pH = 7.6). An important deduction from a study of oxygen diffusion in the frog's auricle and ventricle is that tissue more than 0.2 mm. thick will not allow of adequate diffusion of oxygen if suspended in air and may not when suspended in oxygen receive an adequate supply of the gas. This cuts deeply at all experiments on suspended strips of cardiac tissue where no accurate measurement of the thickness of the strip has been made. Particularly is this criticism levelled at ventricle strips. The proof of the value of using auricular tissue is seen in the fact that an auricle filled with a Ringer-

serum mixture maintains a full efficiency as seen from record of rate and height of contraction for 24 hours, whether it be suspended in air or oxygen. On the other hand while an empty auricle will contract efficiently in oxygen it begins to fail within two or three hours if suspended in air. The rate of metabolism of the arrested ventricle of the frog lies between 20 and 30 per cent of the rate of metabolism when the ventricle is beating at 15 per minute. There is a definite linear relation between the metabolic rate of the frog's heart and its frequency up to 30 beats per minute (Clark, 1935). The figures given by several workers for the oxygen consumption of the frog's heart as a whole or of its chambers vary greatly. Individual variations are so great that they fail to show any relationship between the size of the heart and its metabolism. Such variations as are seen in the following table are undoubtedly due to differences in method, and such figures would certainly assume new interpretative values were they all accompanied by figures indicating the reaction of the perfusion fluid, the amount of CO_2 and lactic acid produced, the diastolic volume and the work done by the muscle.

Oxygen consumption calculated in cc./g./hour for the whole heart, ventricle and auricle of the frog

Perfusion fluid	*O_2 utilized cc./g./hr.*	
Whole heart Ringer's solution	1.33	Clark and White (1928)
Whole heart Ringer's solution	1.07	Clark, Gaddie and Stewart (1931)
Whole heart serum plus Ringer's solution	2.13	Clark and White (1928)
Whole heart Ringer's solution	0.59	Fukuda and Naito (1927)
Ventricle Ringer's solution	0.87	Clark and White (1928)
Ventricle serum plus Ringer's solution	0.27	Clark and White (1928)
Ventricle ox red blood corpuscles	2.92	Weizsäcker (1911)
Ventricle with serum	0.81	Weizsäcker (1912)
Ventricle with serum	1.15	Weizsäcker (1912)
Ventricle with serum	1.19	Gottschalk (1913)
Ventricle with serum	2.22	Luscher (1920)
Ventricle with serum	2.54	Scheinfinkel (1926)
Ventricle with serum	1.07	Clark and White (1928)
Ventricle (tortoise) Ringer's solution with blood	1.05	Stella (1931)

The R.Q. of the cold-blooded heart. The work on the cold-blooded heart referred to also indicates that serum or plasma added to Ringer's solution increases oxygen but does not affect sugar consumption. The presence of serum appears to be necessary for the action of sugar in

raising the R.Q. Sugar added to Ringer's solution does not change the amount of oxygen utilized; it merely changes the type of metabolism more and more from protein and/or fat to carbohydrate, a change which may amount to 45 per cent of the total metabolism. The extremely small amounts of sugar and oxygen estimated make it rather difficult to accept Clark's statements that while sugar added to Ringer's solution is removed from the perfusing fluid it does not affect the R.Q. of the frog's heart, when the figures given are, for Ringer alone, R.Q. = 0.80, for Ringer plus 0.1 per cent glucose, R.Q. = 0.87. In one series of experiments oxygen is less than the control figure, in another series of six hours' experiments it is higher, a fact which is regarded as "probably accidental." What is required is a more accurate estimation of O_2 and CO_2 and it is probable then that one would conclude that oxygen does not change while the CO_2 and R.Q. do increase. Another result of importance is the failure of insulin to raise the R.Q. of the frog's heart beyond 0.9 from about 0.85; the isolated heart of the frog apparently is benefited neither by sugar nor by insulin. These results are in accord with the findings of Rothschild (1930) which indicate the inability of minced and therefore greatly damaged skeletal muscle of the summer frog to alter its oxygen consumption upon the addition of glucose or insulin, although the winter frog muscle does appear to utilize sugar. The general conclusions are, that the frog's heart has a limited power to use carbohydrates, that the presence of serum, glucose, and insulin has little effect in increasing carbohydrate metabolism, which never under most favorable circumstances exceeds 45 per cent of the total metabolism, that protein is used for energy and for glycogen formation and that the type of metabolism resembles that described by Needham (1927) for amphibian embryos which utilize proteins, fats and carbohydrates in the percentage ratio of 71, 22 and 7. It is remarkable, however, that in this work of Clark's, which is the most important contribution to date on the study of the metabolism of the frog's heart, such definite results have been obtained in face of the numerous difficulties which are invariably associated with the use of such small pieces of tissue in rather an abnormal environment showing extremely small changes in gaseous metabolism.

Lactic acid production in the cold-blooded heart. The failure to recognize the rôle of lactic acid in muscle efficiency led to several rather abstruse conclusions from results obtained by immersing the frog's ventricle in oxygen-free saline. Diminution in ventricular contraction under such conditions was early demonstrated by Howell (1901) and

Lingle (1902, 1905). The first observer to note the favorable effect of alkali on the heart's action was Merunovicz (1875), whose findings were confirmed by Gaule (1878) and Benedict (1905). In 1905 E. G. Martin assumed that the failure of the exhausted and asphyxiated heart was due, not to oxygen-lack but to the fact that the ability of the tissue to absorb oxygen was diminished because "something is lost by the tissue upon which its power to absorb oxygen depends." The power to utilize oxygen was restored by the addition of calcium and alkali (NaCl). The explanation was soon forthcoming. Fletcher and Hopkins (1907) in their classical researches on lactic acid production showed that in amphibian muscle a definite amount of lactic acid was produced on stimulation of the muscle to fatigue and that larger amounts were obtained when the muscle passed into rigor. That the anaerobic production of lactic acid was accompanied by an equivalent loss in glycogen of the muscle was first demonstrated by Meyerhof (1920, 1921). If the muscle be suspended in an alkaline phosphate solution all the glycogen becomes converted into lactic acid. This holds for skeletal but not for cardiac muscle, for heart muscle is much more dependent upon a continuous oxygen supply than is skeletal muscle. The anaerobic activity of the isolated frog's heart depends upon two independent variables, namely, the amount of carbohydrate available for use by the heart and the reaction of the perfusion fluid (Clark *et al.*, 1932). In contradistinction to aerobic activity the heart, under anaerobic conditions, utilizes carbohydrate, the essential source of its energy being the breakdown of carbohydrate to lactic acid. The many contradictory results obtained by several workers on the production of lactic acid by the heart are undoubtedly due to varying degrees of lack of oxygen. The extreme figures obtained by Nagaya (1929) and Eismayer and Quincke (1930) from almost zero to 4 mgm./g./hour of lactic acid, can be explained by the method of perfusion adopted. A double cannula affording a flow of well oxygenated fluid through the heart from sinus to aorta will give zero figures, a Straub cannula will produce the anoxemic figures. Wertheimer's (1930) work on ventricular strips of frog's heart showed that in the presence of a rich supply of oxygen, the greater the work done the less the amount of lactic acid excreted by the heart into the perfusing fluid. In six cases of poisoning with NaCN·M/2000 to M/3000, control strips showed an excretion of 0.166 mgm./g. and a lactic acid content of 0.010 g./100 g. of muscle, the poisoned muscle strips gave an average excretion of 0.85 mgm./g. of muscle and contained on an average of 0.037 g./100 g. of muscle.

Anaerobic metabolism of the cold-blooded heart. The effect of oxygen-lack on the lactic acid content of the cold-blooded heart has been investigated by many (Redfield and Medearis, 1926; Arning, 1927; Gemmell, 1928; Boyland, 1928, etc.). Lactic acid is rapidly excreted in the poisoned muscle; it is also rapidly excreted in the anoxemic muscle, but the degree of excretion is dependent upon the alkalinity of the perfused fluid. By removing the lactic acid the alkalinity of the fluid antagonizes somewhat the effect of oxygen lack (Martin, 1905; Drury and Andrus, 1924). If the Ringer's perfusion fluid be unbuffered the frog's heart will cease all activity within an hour. This result was formerly thought to be due to the accumulation of lactic acid within the heart muscle, but in 1933 Gottdenker and Wachstein suggested that asphyxial arrest of the isolated rabbit's auricles was possible without any appreciable increase of lactic acid within the muscle. As a result of this finding Clark *et al.* (1934) repeated their experiments upon asphyxial arrest of the frog's ventricle and concluded that the decreased power of the heart to maintain anaerobic activity was due to a decrease in its power to buffer the lactic acid formed. It would appear that of the lactic acid produced by the heart, part is retained and part is passed into the perfusing fluid as free acid and as lactate. The frog's ventricle contains sufficient potassium and sodium to neutralize all the lactic acid that may be formed in the heart muscle. In asphyxia of short duration the frog's heart may produce and neutralize 1.5 mgm. lactic acid per gram of muscle, changing the pH from 7.5 to 6.5; further asphyxia results in a production of free lactic acid showing that the availability of base and therefore the buffer capacity of the heart is limited (Furusawa and Kerridge, 1927). The buffering power of the frog's heart is stated to be about half that of the frog's skeletal muscle (Brody, 1930). The essential cause then of asphyxial failure is not the accumulation of lactic acid in the heart muscle. It is probable that the increasing acidity of the perfusing fluid in the absence of oxygen inhibits the anaerobic production of lactic acid which is necessary for the supply of energy for the restoration of creatine phosphate.

The effect of glucose on the anoxemic heart. That hearts perfused with Ringer's fluid containing glucose can beat actively for many hours under anaerobic conditions has been demonstrated (Backmann, 1927; Freund and König, 1927; Clark *et al.*, 1932). The anaerobically fatigued heart can be revived by the addition of glucose to the perfusing fluid provided that the fluid is alkaline. While the anaerobic frog's

heart without glucose in the perfusion fluid uses its own glycogen almost exclusively, the nitrogenous metabolism being very much lower than under aerobic conditions, the aerobically active heart uses no circulating sugar; its metabolism consists of approximately 40 per cent each of carbohydrate and protein leaving 20 per cent undetermined. The question of the production for energy needs of carbohydrate from unknown sources has not been settled. Clark *et al.* (1932) maintain that inositol is not used by the frog's heart. Boyland (1928) favors the production of lactic acid from inositol in the heart. It has been frequently stated that inositol improves the activity of the heart of the frog better even than glucose; for example, Hewitt and De Souza (1921) found that small amounts of inositol increased the force of contraction but not the rate of the perfused heart. Chevalier and Brissemoret (1908) found a similar improvement in the rabbit's heart. Sachs (1906), on the contrary, found that inositol led to systolic arrest of the heart; these results, however, may have been due to toxic doses of the drug. The methods for the estimation of inositol are evidently at fault; that devised by Needham (1923) cannot be regarded as specific.

As a means for the study of possible intermediary sources of energy in carbohydrate metabolism, the exhausted frog's heart beating anaerobically in Ringer's fluid through which nitrogen is bubbled has led to certain noteworthy conclusions (Gaddie and Stewart, 1934). When nitrogen is bubbled through Ringer's fluid of the following percentage composition, NaCl 0.65, KCl 0.015, $CaCl_2$ 0.012, $NaHCO_3$ 0.05, the pH of the fluid is raised to 8.5. Hearts perfused with such a fluid are exhausted in 2 to 6 hours, the time depending on the amount of glycogen present in the heart, the rate of stimulation and the frequency with which the fluid is changed. According to Gaddie and Stewart the fluid washes out a factor which is essential for proper contraction of the heart and the more frequently the fluid is changed the more rapid is the exhaustion. Such an exhausted heart was found to be easily restored by addition of glucose and mannose. It was not restored by fructose, galactose, the *pentoses* arabinose, ribose, xylose and the *disaccharides* glycogen, and starch. Such results corroborate, in part, work carried out to determine the ability of the mammalian heart to utilize these carbohydrates. Maclean and Smedley (1913) found that the hearts of dogs and rabbits utilized glucose and mannose well, galactose to a less extent and maltose, fructose and xylose not at all. Steinberg (1927) found that the rabbit's heart did not utilize fructose. It would appear that the utilization of added glycogen by the heart

described by Freund and König (1927) was the result of the presence of traces of glucose in the glycogen used. The question of the part played by the intermediary products of carbohydrate metabolism in heart activity will be discussed later when the gaseous metabolism of the mammalian heart has been reviewed. It is sufficient at present to state that methyl glyoxal produces a partial recovery of the frog's exhausted ventricle, pyruvic acid, sodium glycerophosphate and dihydroxyacetone are separately without effect. Pyruvic acid and sodium glycerophosphate together produce a slight recovery of the exhausted anaerobic heart.

The action of iodo- and bromo-acetic acid. The anaerobic ventricle is rapidly stopped by iodo- and bromo-acetic acids in concentrations of 0.25 millimol. Oxygen rapidly revives such a heart if given at once. Gaddie and Stewart (1934) have also shown that monochloracetate, in concentrations up to 2.5 millimols, has no effect upon the heart and they emphasize the importance of this fact in view of the observations of Dudley (1931) that iodoacetic acid inhibits glyoxalase, of Jowett and Quastel (1933) that iodoacetic acid inactivates the co-enzyme of glyoxalase indicating that this co-enzyme is glutathione, of Dickens (1933) that *in vitro* iodoacetic acid reacts with glutathione liberating hydriodic acid.

The action of glutathione. Mowat and Stewart (1934) showed that glutathione abolishes the power of iodoacetic acid to inhibit glycolysis in blood. Gaddie and Stewart (1934) found that glutathione is unable to restore to full power the ventricle which has been poisoned by iodoacetic acid, but restores fully the ventricle which has been poisoned under *anaerobic* conditions by means of a minimal dose of trivalent arsenic (0.25 millimol sodium arsenite). It appears that the arsenic and iodoacetic acid specifically inhibit enzymes responsible for the breakdown of carbohydrate to lactic acid and in recovery by glutathione it has been noted that the frog's ventricle behaves differently upon the addition of glucose to the perfusion fluid according to whether it has previously been poisoned by iodoacetic acid or sodium arsenite. In the former case glutathione, probably destroyed by iodoacetic acid, is made good but the recovery is never complete. The addition of glucose in the presence of glutathione stops the heart previously poisoned by iodoacetic acid. The question arises, is there a toxic intermediate substance, formed and maintained in the presence of iodoacetic acid and removed by glutathione? Gaddie and Stewart suggest glyceric aldehyde. In the latter case, in the absence of oxygen, sodium arsenite

(1:50,000) inhibits glyoxyalase upon the activity of which anaerobic carbohydrate contraction depends (under stronger doses of arsenic the heart will stop even if given oxygen, showing that it is then acting as a general enzymic poison). The addition of glucose does not restore the heart but the addition of glutathione does and further it maintains cardiac activity until all carbohydrate stores are exhausted. The further addition of glucose will then maintain activity. Since according to Meyerhof (1933) the production of lactic acid from glycogen by way of triose phosphates and pyruvic acid is independent of glutathione, it would appear probable that methyl glyoxal may be the main intermediate in the production of lactic acid from glucose in the cardiac muscle of the frog. That methyl glyoxal can restore the anaerobically exhausted heart, that glutathione is the co-enzyme of glyoxylase, that iodoacetic acid reacts with and destroys glutathione (Dickens, 1933) and that trivalent arsenic combines with glutathione (Voegtlin *et al.*, 1931) would tend to support this contention.

THE GASEOUS METABOLISM OF THE MAMMALIAN HEART. Since the work on the mammalian heart has been carried out either on the isolated heart-lung preparation or on the excised heart perfused with Ringer-Locke's solution or with defibrinated blood, brief reference to one or two points in method upon which certain criticism bears may be permitted. Open or closed systems of blood perfusion lead to a loss of CO_2 from the blood and it is well to know the extent of such loss under the experimental conditions obtaining. It has been found that during the 20 to 30 minutes taken for the preparation of the isolated heart-lung, the opening of the chest, the filling of the venous blood reservoir with blood which has been defibrinated and frequently filtered, there is a marked loss of CO_2 from the blood. The loss of blood CO_2 during the first half-hour following the completion of the isolated heart-lung preparation may be as much as 25 per cent, and during the second half hour about 15 per cent. Equilibrium of blood CO_2 can usually be attained within 60 minutes from the time of completion of the preparation (Cruickshank, 1934). Any experimental data necessary for R.Q. determination should always be corrected, if taken within such a time limit. Such gaseous exchange through the pleura as has been noted by Kremer, Wilson and Wright (1934) can be prevented by closing the chest and allowing but the smallest possible contact of air with the apical visceral pleura. Artificial respiration must be regulated as far as can be judged to meet the requirements of the active heart muscle, the weight of which can be easily judged. In a

dog of 10 kgm. weight with a heart weighing 50 grams the respiration rate should be about 12 per minute and the stroke volume approximately 100 cc. It is also important in using closed respiratory systems in the determination of gaseous metabolism frequently to apply alcohol lamp checks. Data concerning such checks should always be expected in the description of new methods. In using the excised mammalian heart perfusion with any fluid except the blood of the animal from which the heart is taken is open to criticism. No mammalian heart can be regarded as approaching the normal in its activity if deprived of its hemoglobin, if under conditions which would alter the calibre of its coronary vessels, and if prevented from developing the physiological initial length-tension of its muscle fibres. These normal requirements never obtain in the excised heart perfused by way of the aorta whatever be the perfusing fluid. The frog's heart so perfused may virtually answer to these requirements, the mammalian heart never: it must be examined *in situ* and the nearest approach to normal conditions at present is found in the isolated heart-lung preparation. An adequate account or discussion of the energy metabolism of the heart cannot be forthcoming until all the biochemical events involved are clearly set out. It is only recently in investigations concerning the gaseous metabolism of the heart that an attempt has been made to differentiate between the utilization and storage of carbohydrates, to estimate simultaneously the CO_2 produced and oxygen used, to consider the metabolism of the lungs as part of that of the whole heart-lung preparation and to assess as accurately as possible the physiological condition of the heart throughout the experiment. This has been attempted in the excised mammalian heart (Soskin and Katz, 1932), in the isolated heart-lung preparation (Cruickshank and Startup, 1933a) and in the heart-lung preparation with and without the use of an oxygenator system (Evans et al., 1935). Present-day problems of the chemistry of muscle contraction are sufficiently complex to make one realize how much is still demanded of physiological investigation of the metabolism of cardiac and skeletal muscle. It is clear that only some of the main lines of investigation and the results secured can be touched upon.

Utilization of glucose by the mammalian heart. The question of the utilization of carbohydrate by the mammalian heart has been obscured by the lack, in certain investigations, of suitable estimations of changes in the lactic acid content of the blood, of a knowledge of the irregular variations in glycolysis in the circulating blood and of the utilization of sugar by the lungs. Were all the factors known and corrections

duly made, there would possibly be far less disagreement in the general average of results published. A comparison of some of the figures for the utilization of carbohydrate by the heart will show how great are the variations. In 1913 Patterson and Starling found the glucose loss of the heart-lung preparation to be from 0.8 to 3.8 mgm./g./hr. Cruickshank and Startup (1933a) gave an average figure of 5.28 mgm./g./hr. with a variation between 4 and 6 mgm./g./hr. McGinty and Miller (1933) found by two different methods 0.2 and 0.3 mgm./g./hr. Lovatt Evans and his fellow-workers (1933) gave an average figure of 0.072 mgm./g./hr., their highest figure being 2.4 mgm./g./hr. They point out that in preparations made acapnic by ventilation with air, the glucose usage of the heart is practically negligible. Such variations indicate a variety of causative factors. The part played by the lungs has been investigated, and it has been shown that the heart uses much less sugar directly from the blood than has been previously supposed (McGinty and Miller, 1933; Rühl, 1934; Evans, Grande and Hsu, 1935). Evans, Hsu and Kosaka (1934) showed that the lung tissue, corresponding to 100 grams of heart, causes the average disappearance of 113 mgm. of glucose in the first hour of perfusion. Of the sugar which disappears from the blood not all is oxidized in the lungs, much is converted into lactic acid; the fraction so converted is less than in the blood itself and is stated to be about 46 per cent and 82 per cent in the first and second hours respectively. It is clear that glucose is lost from the blood in passing through the lung, the results of Patterson and Starling (1913), Cruickshank and Startup (1930) and Hemingway and Phelps (1934) average 81 mgm./100 g. heart/hour.

Fallacies in the estimation of glucose consumption by the heart certainly arise from incorrect allowances for glycolysis both in the lungs and in the controls. Hemolysis is always greater in oxygenation than in lung-perfusion circuits. It may be thought that the slower rate of glycolysis in the oxygenator circuit is due to corpuscular damage (Irving, 1926; Reid and Narayana, 1931) but the fact remains that in the lungs and in the perfusing blood of the isolated heart-lung preparation, lactic acid is formed and is or may be oxidized by the heart. Herein lies a valid criticism of those results which attribute to the heart an oxidation of sugar of the order of 5.00 mgm./g./hr. with a R.Q. of unity. The part played by the lung may under variations of hyperglycemia be much more than 1.13 mgm. per gram of heart muscle per hour. Corrections for glycolysis bring about a good agreement be-

tween the results of Evans and Cruickshank. The former has shown that adding the oxidation due to glycolysis to that for glucose consumption of the heart, the total amounts of glucose caused to disappear in the isolated heart-lung preparation average 4.20 (Evans) and 5.28 mgm./g./hr. (Cruickshank). Hyperglycemia undoubtedly leads to a deposition of glycogen in the heart and the extent of this synthesis has been assumed by Cruickshank and Startup (1933a) to be the difference between the sugar equivalent of the oxygen burned by the heart and the total amount of glucose disappearing from the blood. The degree of synthesis, for reasons already mentioned, cannot be so determined. The synthesis with a R.Q. of unity would be greater than their figures suggest. In hypoglycemia, i.e., with the blood sugar reduced to 0.03 gram per cent in one hour, the utilization of blood sugar by the heart falls steadily with an accompanying loss of heart glycogen. It has been shown that when the R.Q. falls below 0.85 there may be but little utilization of carbohydrate by the heart and in aglycemia with a R.Q. = 0.7 and a loss of cardiac glycogen of about 20 per cent, no carbohydrate is burned by the heart (Cruickshank and Startup, 1933b). That the heart well retains or loses but sparingly its glycogen under conditions of work and of hypoglycemia lasting from three to five hours is generally accepted. Visscher and Mulder (1930) give the following figures which have been corroborated by many; for the normal heart unworked, glycogen is 0.560 gram per cent with a standard deviation of ± 0.165 and a probable error of the mean of ± 0.022; for worked hearts, the mean is 0.516, the standard deviation ± 0.133 and the probable error of the mean ± 0.023. The question of the utilization of sugar and deposition of glycogen by the lungs requires investigation. It would therefore appear that the results generally found for glucose utilization are too high, because the most recent work (Evans, Grande and Hsu, 1935) would indicate that the lungs perfused *in situ* are capable of converting a very appreciable amount of glucose into lactic acid. Whether or not lactate will accumulate in the blood of a heart-lung preparation depends upon the volume of blood, the size of the heart and the type of lung ventilation. Ventilation of the lungs with 5 to 10 per cent CO_2 mixtures causes a fall in the pH of the blood which, by retarding glycolysis, tends to a reduction of blood lactate. Given a normal heart of about 100 grams with a total blood volume in perfusion of 800 cc. it may be said that 114 mgm. of lactic acid will be formed by glycolysis per hour, the heart will remove 234 mgm./hr. resulting in an hourly loss of 120 mgm. of lactic acid, which is equiva-

lent to a fall of 15 mgm./100 cc./hour. Out of a great number of varying results, this perhaps sums up the situation and indicates the factors, the interplay of which results in a normal balance between glucose and lactic acid in the blood under such experimental conditions. It is clear when discussing carbohydrate metabolism of the heart that oxygen determinations should be regarded as the sum of glucose and lactic acid burned. When the sum of glucose and lactate is taken, and since they have the same formulae and a common metabolic history they should so be taken, the fluctuations so common to glucose estimations become greatly reduced. Rühl (1934), Evans *et al.* (1934) and others have shown that there is a definite relation between the concentration of these substances and their uptake by the heart. Plotting the usage in milligrams per 100 grams of heart per kilogram in work done against the concentration of each constituent, Evans has shown that the relation is a closer one for glucose than for lactic acid and he has stated that there is ample room for speculation as to what factors there may be other than the concentration of the constituent. A general conformity of results is seen by summing the uptake by the heart of these two substances and noting the average figures for the oxygen utilization of the heart-lung preparation from three different laboratories. Starling and Evans (1914) found the oxygen utilization to be 3.24 cc., Cruickshank and Startup (1933a) 3.96 cc. and Evans, Grande and Hsu (1934) 2.82 cc./g.hour. Discrepancies are further reduced when the average duration of the experiment is noted, namely, 1.4, 1.0 and 2.0 hours respectively, for, as the blood sugar falls, the heart quickly resorts to other sources for its energy needs.

The R.Q. of the mammalian heart. Unless an accurate respiratory quotient of the heart is determined, it is impossible to determine the partition of oxygen utilized between the possible sources of energy, carbohydrate, protein and fat. Experiments upon the gaseous metabolism of the excised perfused heart, using the methods of Langendorf or Heymans and Kochman are open to so much criticism that they need not be referred to here. A certain amount of controversy has arisen over the question of the validity of R.Q. determinations particularly in eviscerate preparations. It is frequently suggested that the observed R.Q. is not reliable as a guide to the nature of oxidative metabolism because of the variations in the CO_2 output which may occur from changes other than those of oxidation (Verzár, 1911; Murlin, Edelmann and Kramer, 1913; Krogh, 1923; Kilborn, 1928; Kilborn, Soskin and Thomas, 1928; Bornstein, 1929; Ferguson, Irving and

Plewes, 1930; Anderson, Cleghorn, Macleod and Peterson, 1931). It is known that overventilation will cause an increase in lactic acid (Eggleton and Evans, 1930) and it has been demonstrated that there may be a very close relation between CO_2 displaced from combination in the blood by lactic acid formation and the respiratory quotient (Peterson, 1933). It is, however, maintained by some that a respiratory quotient of unity can be secured in the eviscerate preparation if overventilation be excluded and if the blood sugar be maintained at the normal content (Bornstein, 1929; Corkhill, Dale and Marks, 1930). With regard to the R.Q. of the isolated perfused heart, it is essential if respiratory quotients of unity are to be obtained, that the blood sugar be maintained and the pulmonary ventilation be minimal (Cruickshank, 1934). The early experiments which aimed at determining the R.Q. of the heart (Evans, 1912–13; 1913–14; Starling and Evans 1914–15) showed variations, during experiments of from one to five hours' duration, from unity to 0.60 with an average of 0.85. What was shown was that much depended upon the previous feeding of the animal, that glucose raised the R.Q. of the heart, if not to, at least towards unity and that the heart did not rely entirely on carbohydrate for fuel. Of the recent work, that of Cruickshank and Startup (1933a) would indicate that a R.Q. of unity can only be expected in the presence of an adequate supply of glucose. Where the blood sugar is allowed to be depleted by the actively beating heart, the R.Q. begins to fall when the blood sugar is approximately less than 50 mgm. per cent. Where no glucose is added to the circulating blood and where one or more hours are allowed to pass before estimating the R.Q., then it will never be found to be unity (Bayliss, Müller and Starling, 1928; Cruickshank and Startup, 1933a, b). Progressive hypoglycemia results in a fall in CO_2 production with practically no change in oxygen utilization, and therefore a fall in the R.Q. The rate of fall of the R.Q. depends upon the original blood sugar, the extent to which glycogen may be utilized and the activity of the heart. Heart glycogen is not readily lost (Visscher and Mulder, 1930). With a fall of blood sugar to 50 mgm. per cent oxygen utilization is reduced from 3.96 to 3.00 cc./g./hr. In a period of 2 to $2\frac{1}{2}$ hours during which the blood sugar has disappeared, the CO_2 production may show a 50 per cent fall, the oxygen a 20 per cent fall and the R.Q. will be between 0.68 and 0.71. If the aglycemic condition be produced rapidly by the addition of 5 units of insulin half-hourly to the perfusing blood, the R.Q. will be at or below 0.70 within 30 to 45 minutes depending upon the

size of the heart, the blood volume, the original blood sugar content, and the degree of oxygenation of the blood. It has been shown that in insulin or adrenaline aglycemia (Cruickshank and Startup, 1933b; Cruickshank and McClure, 1936) there is no loss of cardiac glycogen, that in 2 hours oxygen utilization may rise from 5 to 8 per cent and CO_2 production fall 30 to 35 per cent while the R.Q. is at 0.7 within an hour. Such results bear out the fact, which holds for the frog's heart as well as the mammalian, that sources, other than carbohydrate, must be immediately available for cardiac activity and that under physiological conditions carbohydrates may be utilized in proportions of the total sources of energy varying from 40 to 100 per cent.

The action of insulin on the gaseous metabolism of the heart. That the glucose utilization of the heart is increased by insulin is the opinion of a majority of workers (Hepburn and Latchford, 1922; Burn and Dale, 1924; Cruickshank and Startup, 1933a; Cori and Cori, 1926; Evans, Grande and Hsu, 1935); a minority states that there is either no change or a diminution (Plattner, 1924; Peserico, 1926; Visscher and Müller and Starling, 1927). According to the hypothesis of Evans, supported by the work of Cook and Hurst, lactic acid produced by glycolysis in the blood is taken up by the heart, skeletal muscle and liver. In the heart-lung preparation the lactic acid is most probably oxidized, or, what is less likely, it replaces an equivalent amount of glycogen which is oxidized. The mechanism of the production and utilization of lactic acid in heart-lung preparations is seemingly not affected by insulin. It would appear from the work of some (Cruickshank, *et al.*, 1933a) that the characteristic action of insulin on the heart is the synthesis of glycogen. This is in agreement with the work of many on insulin action (Best *et al.*, 1926; Burn and Dale, 1924; Cori *et al.*, 1923; Bissinger, Lesser and Zipf, 1923). Insulin may exert a very slight pressor effect upon the heart, 12 to 20 mm. Hg for 5 or 10 minutes, but such action is of very short duration and any increase in oxidation that occurs cannot be entirely attributed to pressor effects. Visscher and Müller (1927) using the method for oxygen estimations described by Starling and Visscher (1926–27) found that where there was no increase in heart rate and where the ventricular diastolic volume did not alter appreciably there was no change in oxygen consumption upon the injection of insulin. It would appear that even where there is an excess of carbohydrate in circulation the utilization of oxygen by the heart can never be increased more than 3 to 4 per cent (Cruickshank and Startup, 1933a). Insulin (Eli Lilly) never shows pressor effects on the

pithed cat (K. K. Chen, 1934). Its effect, therefore, in the metabolism of the heart is to increase the deposition of glycogen to withdraw carbohydrate from the circulating blood and so quickly alter the type of metabolism from carbohydrate to fat. This change is equicaloric, which is of interest in view of the finding of Cori and Cori (1928) in their work on the disappearance of carbohydrate in the post-absorptive state in rats, that a change in R.Q. from a normal of 0.82 to 0.94 by insulin injection is accompanied by a slight fall in oxygen used and a 14.8 per cent increase in CO_2 produced.

Utilization of fat. The question of the utilization of fat by the heart demands a great deal of investigation before any definite conclusions can be arrived at. By a process of elimination many have decided that muscle activity is dependent upon energy sources other than carbohydrate. The evidence on the question of the direct oxidation of fat by skeletal muscle is contradictory; negative evidence is given by Leathes (1906) and Winfield (1915), positive evidence by Lafon (1906), Palazzola (1912), Benedict and Cathcart (1913), Krogh and Lindhard (1920) and Takane (1926). Investigations upon the R.Q. of resting and contracting muscle and the sartorius muscle poisoned with iodoacetic acid would indicate the ability of this tissue to burn fat (Himwich and Castle, 1927; Himwich and Rose, 1929; Meyerhof and Boyland, 1931) and would also show that fat is undoubtedly utilized in exercise (Zuntz, 1911; Anderson and Lusk, 1917; Hill, Long and Lupton, 1924; Rapport and Ralli, 1928; Rapport, 1929 and Gemmell, 1934). Such results would make it not improbable that the heart should be able to burn fat. The question, like many others, must be decided by work on the mammalian heart. The hearts of dogs previously fed for several days on a diet consisting largely of butter, generally show very low respiratory quotients (Evans, 1914). The fact that in isolated perfused hearts where the blood sugar has been reduced almost to zero, respiratory quotients of 0.7 have been obtained over periods of one to two hours' duration, is indicative of the ability of the heart to burn fat. As Cruickshank and Startup say of the aglycemic heart, "when a heart maintains a rate of 160 beats per minute, a blood pressure of 90–100 mm. Hg for three hours with not a trace of blood sugar and an R.Q. varying between 0.72 and 0.68 with an average over five 30-minute periods of 0.699, then there is no question of the ability of the isolated heart muscle to utilize fat." From further investigation upon the R.Q. of the diabetic heart, it would seem that this is a direct oxidation of fat and not a burning of fat by way of carbohydrate. In an attempt to correlate fat and amino-acid utilization, Cruickshank and McClure

(1936) have failed to secure evidence of amino-acid utilization and since the preparation is aglycemic, shows no evidence of any derangement of fat metabolism as indicated by the diminution of acetone bodies in the blood and the loss of fat from the heart muscle, they suggest that in so far as deductions may reasonably be drawn from their results the balance of experimental evidence is in favor of a direct burning of fat by the cardiac muscle.

Gaseous metabolism of the diabetic heart. Numerous investigators have shown that the totally depancreatized animal has a respiratory quotient of 0.7 (Starling and Evans, 1914–15). The heart of such an animal is either oxidizing fat directly or converting fat into carbohydrate and burning it as such. Much controversy has arisen round the question as to what is the precise metabolic error responsible for diabetes. A transformation of fat into carbohydrate was regarded by Lusk as a superstition, while the possibility of such transformation and the formation of intermediary bodies of importance in carbohydrate metabolism was tenaciously maintained by Macleod. One cannot here discuss the question as to whether the essential feature of diabetes is a failure of the organism to oxidize sugar or an over-production of carbohydrate from fat, but one may briefly look at the evidence which more recent work on the diabetic heart supports and try to decide to what extent such evidence on the isolated heart may be valid as a contribution to the solution of this vexed question.

It is a curious and unexplained fact that the diabetic heart has a glycogen content 30 to 50 per cent greater than the normal; average figures may be taken as 0.60 and 1.25 g./100 g. for normal and diabetic hearts respectively (Knowlton and Starling, 1912; MacLean and Smedley, 1913; Cruickshank, 1913; Kosterlitz, 1933; Cruickshank and Startup, 1934). The high figures recorded for the glycogen content of the diabetic heart, would tend to the conclusion that the heart had lost completely the power of utilizing its reserve carbohydrate but not the power of synthesizing glycogen. A consideration of how the diabetic heart (i.e., from a dog, seven days' diabetic) responds to insulin, reveals certain clean cut facts. It is seen that upon the addition of 10 units of insulin, the respiratory quotient is rapidly raised from 0.7 toward unity. This is associated with a 30 to 40 per cent increase in CO_2 production and a 5 per cent increase in oxygen consumption. The significance of such changes is best seen from an example. A normal heart-lung preparation in hyperglycemia has an oxygen consumption of 4.4 cc./g./hour: a diabetic heart-lung with R.Q. = 0.71 has an oxygen consumption of 5.00 cc./g./hour. The

addition of insulin raises the R.Q. to unity within one hour and the oxygen utilization is changed from 5.00 to 5.14 cc./g./hour. Of an entirely different order are the changes in CO_2 production. For this change in the R.Q., the CO_2 production has shown a 40.6 per cent increase, from 3.62 cc. to 5.09 cc./g./hour. Such results are only secured in dogs which are markedly diabetic and dogs are not markedly diabetic less than 6 or 7 days after depancreatectomy. The experimental results and conclusions of Yater, Markowitz and Cahoon (1933) are open to criticism. There is indeed no foundation for the assumption that the utilization of sugar by the resting skeletal muscle is comparable to that of the diabetic muscle, skeletal and cardiac, for it is known that the isolated heart-lung progressively loses its power to burn sugar as the preparation is made from animals from two to nine days after total depancreatectomy (Cruickshank and Shrivastava, 1930). There is, however, no loss of power on the part of the diabetic heart to utilize lactate (Evans, Grande, Hsu, Lee and Mulder, 1935). From such results it is fair to conclude that there is under insulin a rapid equicaloric shift from fat to carbohydrate in the metabolism of the diabetic heart. It is a shift suggested by many as a usual result of insulin in experimental animals (Dale, 1923, Krogh, 1923; Minkowski, 1924; Macleod, 1924, 1926). A similar shift has been described for diabetic patients by Fitz, Murphy and Grant (1922). Is the change in the metabolism of the diabetic heart due to restoration of carbohydrate oxidation or the cessation of the over-production of carbohydrate from fat? If it were the former we should expect 1, a rise in the R.Q.; 2, an increase in CO_2 production; 3, little or no change in oxygen utilization; 4, an increase in sugar utilization from zero to normal; if the latter, the following events would occur: 1, a rise in the R.Q.; 2, a decrease in oxygen utilization; 3, no change in CO_2 production; 4, an increase in the rate of disappearance of the sugar from the blood, i.e., from a minimal figure up to the normal for the heart. The following figures are self-explanatory (Cruickshank and Startup, 1934).

Weight of heart = 50 grams. Glycogen content = 1.49 grams per cent.
Blood volume = 530 cc.
Dog 7 days diabetic.

PERIOD 30 MINUTES	CO_2	O_2	R.Q.	BLOOD SUGAR REMOVED	SUGAR OXIDIZED	CALS.	INSULIN
	cc./g./hr.	*cc./g./hr.*		*mgm./g./hr.*	*mgm./g./hr.*	*g./hr.*	*units*
1	3.46	4.84	0.71	0.63	0.08	22.70	None
2	4.27	5.12	0.83	4.13	2.80	24.72	5
3	4.88	4.86	1.00	8.01	6.48	24.52	5

The increase in CO_2 production, coupled with the lack of any change in oxygen consumption lends no support to the hypothesis that insulin controls neoglucogenesis from fat in cardiac muscle, but supports the view that the primary defect in the diabetic heart is a failure in the oxidation of carbohydrate. The crucial experiment must, however, be on the whole animal and those results, however interesting they may be do not afford a basis for argument for or against the hypothesis of Macleod (1928) that the low R.Q. in diabetes is due to the excess of oxygen required for the production of carbohydrate from fat and that the function of insulin is to prevent the over-production of carbohydrate from fat.

PHOSPHORIC ACID METABOLISM. The earlier research on phosphoric acid metabolism was carried out without any attempt to relate results to the total metabolism of the heart. It was soon seen that the active heart contained more free phosphorus than the resting one and that the extra phosphorus produced was dependent not on the work of the heart but rather on the rate of contraction. To the fact that the cold-blooded heart could continue its activity without oxygen became added the even more important fact that the phosphoric acid breakdown was an anaerobic process. This breakdown was regarded not as a source of energy but as an essential part of the process of muscle contraction, Wertheimer stating in 1930 that "phosphoric acid breakdown is a measure of anaerobic cardiac activity." In making very accurate comparison of the phosphorus (P_2O_5) content of resting and active strips from the ventricle of the frog, Wertheimer (1930) has shown that the phosphate is increased by activity, and that this takes place under anaerobic conditions. Later, Wertheimer (1931) suggested that during activity the heart resynthesized phosphate in a chemical formation which could be easily broken down and inferred further that activity did not cause any increase in inorganic phosphorus. The researches of Lundsgaard (1930) have shown that the energy for muscular contraction is derived from phosphagen hydrolysis, not from glycolysis, and that phosphagen is synthesized at the expense of glycolysis. Poisoning by iodo- or bromo-acetic acid or by fluoride resulted in a failure of the resynthesis of creatine and phosphoric acid and ultimately led to a disappearance of phosphagen from the muscle. In assessing values to creatine phosphoric acid determinations and in, as is legitimate, making close comparison between cardiac and skeletal muscle, it should be remembered that cardiac muscle contains but one-tenth of the creatine phosphoric acid of the skeletal muscle (Eggleton and Eggleton, 1929). In poisoning by iodo-acetic acid Wertheimer could find no decrease in

the creatine phosphoric acid of strips of the frog's ventricle, either at rest or in action, nor was there any decrease in strips beating for seven to nine hours in Ringer's solution and he doubts therefore the advisability of regarding creatine phosphate as an "activity" substance. But there is no need to expect a diminution of phosphagen in an active heart muscle contracting in oxygen. Wertheimer did not discuss anaerobic activity. The result of researches on the various phosphate fractions in the actively beating heart has been to show that there is essentially no change in these. The conclusion of Wertheimer in 1931 that the creatine phosphoric acid which plays so important a part in skeletal muscle contraction, has no part in the development of energy in the normally beating heart is not now tenable. The interest aroused by the changes in creatine phosphoric acid in muscle was the forerunner of a vast amount of biochemical research into the complicated chemical reactions upon which the oxidation of glycogen or glucose as sources of muscle energy depended. Cardiac muscle metabolism may or may not parallel that in skeletal muscle. Results as far as they at present go would indicate that for frog's heart a parallelism exists. For the actively beating mammalian heart it may not. The differences of opinion upon ammonia production lead one to point to the fact that with the frog's muscle, skeletal and cardiac, all the work has been carried out upon a perfused tissue not in a strictly physiological environment. The relation between carbohydrate and phosphoric acid metabolism has of recent years been extensively reviewed (Fiske and Subbarow, 1925; Meyerhof, 1930; Milroy, 1931; Needham, 1932; Parnas, 1931, 1932; Parnas, Ostern and Mann, 1934). Similar relations in cardiac muscle have not received a like attention. Ostern (1930) has stated that ammonia production is proportional to cardiac activity.

What, one may ask, should be expected to occur in a physiologically active mammalian heart excepting that adenosine triphosphoric acid functions as a phosphorus donator, and adenylic acid as a phosphorus acceptor? (Parnas *et al.*, 1935; Needham and Heyningen, 1935; Lohmann and Meyerhof, 1934.) Following the breakdown of creatine phosphate the liberated phosphorus is accepted by adenylic acid and A. T. P. is formed. Glucose produced by hydrolysis of glycogen is phosphorylated to form fructose diphosphate, from which dihydroxyacetone is formed. Enzyme action results in the formation of phosphoglyceric acid and phosphopyruvic acid from which latter compound the phosphorus is released to be taken up by the adenylic acid. The

A. T. P. again formed donates phosphorus for the resynthesis of phosphagen and so the circle is completed. All the energy arising from these complicated reactions is dependent upon the presence of A. T. P. It is clear therefore that in such an anaerobic cycle neither inosinic acid nor ammonia need be produced. Only in the event of a failure to have a complete resynthesis of A. T. P. would free adenylic acid be present and under such an abnormal condition adenine may be deaminated to hypoxanthine and ammonia and inosinic acid be produced. Further results of the failure of complete resynthesis of A. T. P. would be, a diminution of phosphagen due to the lack of a prompt rephosphorylation of creatine to form phosphocreatine, a much greater depletion of glycogen reserves, an increase in orthophosphate, all of which would be accompanied by a progressive failure of the heart. The rôle of creatine phosphoric acid and A. T. P. as phosphorus donators and adenylic acid as a phosphorus acceptor, in the chain of chemical processes of muscle contraction, is clearly one which concerns the velocity of reaction and the chronaxie of muscle. In conditions of anaerobic iodo-acetic poisoning one would expect the phosphocreatine content of the muscle to fall, the creatine to increase, the A. T. P. to be unchanged and no ammonia to be formed. With excessive fatigue where oxygen debt is marked and where the resynthesis of A. T. P. would fall behind the formation of the products of glycogen breakdown, there would be an increase in lactic acid, creatine and probably ammonia. From the work of Needham and Heyningen on the co-enzyme function of adenylic compounds it is apparent that adenylic acid is the essential factor (P. acceptor) in dephosphorylation of phosphopyruvic acid, that A. T. P. is the donator in the initial stage of glycogen breakdown. The work of Lohmann (1935) on the presence of adenylpyrophosphate and adenosinediphosphate in heart muscle is of interest in this connection.

Phosphagen content of the heart. To determine if the frog's heart (R. Esc. Hungary) beating for twenty-four hours in oxygenated buffered Ringer's fluid showed any change in its phosphorus content, the acid soluble phosphorus was measured in five fractions by Clark, Eggleton and Eggleton (1931) by the method of Eggleton and Eggleton (1929). No change was found, but when oxygen was displaced from the fluid and the tissue by a constant stream of nitrogen there occurred a rapid loss of phosphagen with a corresponding increase in the orthophosphate fraction. The readmission of oxygen caused a quick and complete reversal to the normal amounts. The difficulty of accurately weighing such small amounts of tissue as a frog's heart was somewhat minimized

by expressing the physiological condition of the muscle in terms of the ratio phosphagen P/inorganic P. It has been found that in a normally active heart the sum of these two substances has always remained approximately constant. Experiments performed on the quiescent and active tortoise auricle go to show that the beating heart loses its phosphagen more rapidly than the quiescent heart. While the frog's heart is very susceptible to oxygen lack, it can be kept beating anaerobically for almost an hour if the perfusion fluid be alkaline. Under such conditions there is a diminution but not a complete disappearance of phosphagen and it would appear that both for the ventricle of the frog and tortoise a minimum of 0.55 mgm. of phosphagen P. per 100 grams of heart muscle is sufficient for activity under anaerobic conditions. It is assumed therefore that since this amount of phosphagen is maintained over long periods of oxygen lack that resynthesis of creatine phosphoric acid taking place in diastole keeps pace with its breakdown in systole. Clark, Eggleton and Eggleton (1932) state that the heat evolved per beat of the frog's heart may be taken as approximately 6.5×10^{-3} calories (that is, taking the calorific value of oxygen as 5 calories per cubic centimeter irrespective of foodstuffs burnt and the oxygen utilization of the heart as 1.3 c.mm. per beat per gram of tissue). The resting metabolism of the heart is approximately one-quarter of the metabolism of the heart in moderate activity therefore the heat production due to contraction and relaxation would be approximately 5×10^{-3} calories per beat per gram of tissue. Meyerhof (1930) has given the figure for the heat of hydrolysis of phosphagen obtained by acid hydrolysis as 11,000 calories per g. mol. (31 g. P.). In skeletal muscle the heat of contraction approximates the heat of recovery: the heat of contraction therefore under anaerobic conditions would not be more than 2.5×10^{-3} calories and this would be equivalent to 0.0070 mgm. phosphagen P. hydrolyzed during contraction. This minimal amount of phosphagen is equivalent to 0.70 mgm. per cent in the heart muscle. The phosphagen index under aerobic conditions is for the frog's heart 0.6, for the tortoise 1.0. When phosphagen has been reduced to the minimal amount mentioned the phosphagen index is reduced to variable but extremely low figures, 0.10 to 0.01. Results of a similar kind have been secured by Cruickshank *et al.* (1935) for the mammalian heart. They show that the phosphagen index is an index of the physiological condition of the heart, that in asphyxia and in aglycemia there is a failure of resynthesis of creatine phosphate, that amino-acids, such as glycine, do not prevent the decrease of phosphagen

and that insulin hastens the reduction of the phosphagen index towards zero. It is of interest to note that these findings apply particularly to the mammalian ventricle and not to the auricle.

TABLE 1

Showing phosphagen and orthophosphate mgm./100 g. and the phosphagen index for normal and asphyxiated cardiac and skeletal muscle

TISSUE	CONDITION	PHOSPHAGEN	ORTHOPHOSPHATE	PHOSPHAGEN INDEX	AUTHOR
Frog's skeletal muscle	N.	51.3	14.1	3.60	Eggleton and Eggleton (1929)
	A.	0.0	87.0	0.00	
Tortoise auricle	N.	9.4	9.5	1.00	Clark, Eggleton and Eggleton (1932)
	A.	2.7	17.0	0.16	
Tortoise ventricle	N.	5.0	5.0	1.00	Clark, Eggleton and Eggleton (1932)
	A.	0.6	9.1	0.07	
Frog's ventricle	N.	7.3	11.6	0.63	Clark, Eggleton and Eggleton (1931)
	A.	0.9	12.3	0.07	
Guinea pig's heart	N.	5.2	27.5	0.19	Eggleton and Eggleton (1929)
	A.				
*Cat's heart	N.	6.0	26.7	0.22	Cruickshank, McClure and Mackintosh (1935)
	A.	0.0	52.4	0.00	
*Dog's heart	N.	5.3	27.6	0.19	Cruickshank, McClure and Mackintosh (1935)
	A.	2.6	39.2	0.06	
*Dog's ventricle	N.	4.9	28.0	0.18	Cruickshank, McClure and Mackintosh (1935)
	A.	1.6	39.7	0.04	
*Dog's auricle	N.	5.4	27.7	0.195	Cruickshank, McClure and Mackintosh (1935)
	A.	4.7	27.4	0.171	

N = normal oxygenation. A = asphyxia.
* Unpublished results.

The action of iodo-acetic acid. The loss of power to contract following rapidly upon the addition of iodo-acetic acid to the fluid perfusing a heart under anaerobic conditions results in a final state of permanent contracture associated with a complete loss of phosphagen. The phosphagen index (phosphagen P/orthophosphate P) falls from 0.60 (frog)

or 1.0 (tortoise) to any point between 0.25 and zero. It is emphasized, however, that the loss of mechanical function is not accompanied by a parallel loss of residual phosphagen; only when the heart has ceased to beat does the phosphagen disappear and the disappearance is not complete until the final state of permanent contracture has set in (Clark, Eggleton and Eggleton, 1932). The anaerobically beating heart has failed under these conditions because the iodo-acetic acid has specifically struck at the anaerobic mechanism available for the supply of energy for resynthesis of creatine phosphoric acid. The point of action of iodo-acetic acid and fluoride is at the change from phosphoglyceric acid to phosphopyruvic acid (Embden and Deuticke, 1934; Lohmann and Meyerhof, 1934). As long as this anaerobic process is available the heart will beat, however feebly; when it is not available the heart stops. The aerobic heart has alternative sources of energy for this resynthesis. The loss of phosphagen is accompanied by a parallel loss of mechanical activity if the perfusion fluid is unbuffered and neutral, but the loss in mechanical activity is greatly delayed remaining at 80 per cent of its normal for at least one hour by the use of Ringer's fluid buffered with bicarbonate.

The co-ferment-system in cardiac muscle. The isolation of adenylic acid by Embden and Zimmermann (1927) pointed the way to a thorough investigation of nucleic acid compounds in skeletal and cardiac muscle. In 1929 the barium and silver salts of adenylpyrophosphoric acid from skeletal muscle were isolated by Lohmann, and Fiske and Subbarow respectively. The presence and physiological action of adenine compounds in the heart have been investigated by Drury and Szent-Györgyi (1929–30) and Bennet and Drury (1931). They affirm that the substance which they have isolated from the mammalian heart is identical with and closely related to adenylic acid. They found that the substance isolated had an action identical with adenosine prepared from yeast nucleic acid, that it is inactivated, by removing the inert carbohydrate group (leaving adenine), by withdrawing the amino group (leaving inosinic acid) by placing it in a different position (guanosine) and by changing the phosphoric acid linkage. It is known that adenylic acid is rapidly deaminated by muscle tissue under certain conditions (Embden, Riebeling and Selter, 1928) and it has been shown that muscle adenylic acid is more rapidly deaminated than yeast adenylic acid, adenine, etc. (Schmidt, 1928). Drury has shown that adenylic acid produces heart block in the guinea pig, bradycardia in the dog and cat, lengthens the conduction time between auricle and

ventricle and shortens the absolutely refractory period of the auricle. Pohle (1929) isolated adenylic acid from ox heart muscle (1 g. from 5 kgm. muscle) and stated that this acid was not identical with yeast adenylic acid but was identical with that isolated from frog's skeletal muscle by Embden and Schmidt (1929). In 1931 Lohmann isolated from skeletal and cardiac muscle adeninenucleotidepyrophosphoric acid to which he gave the shorter name adenylpyrophosphoric acid. According to Beattie, Milroy and Strain (1934) the nucleotide of cardiac muscle differs chemically from the adenosinetriphosphoric acid of voluntary muscle, being more closely allied to a dinucleotide composed of one molecule of adenosinediphosphoric acid and one molecule of adenosinetriphosphoric acid. It is known that the glycolytic ferment in voluntary muscle consists of a colloidal ferment and a co-ferment adenosinetriphosphoric acid (Meyerhof and Lohmann). The dinucleotide has been tested as a co-ferment on cardiac extracts from lambs' hearts acting on artificial substrates of starch and glucose with orthophosphate. Experiments by Strain (1934) have shown that fresh extracts with ice-cold saline are active for several hours, but after 24 hours' incubation at 4°C., when there is no further production of esters or of lactic acid, the addition of a small amount of the cardiac dinucleotide or the adenosinetriphosphoric acid of skeletal muscle restores activity. It is noteworthy here that the cardiac dinucleotide has a much more powerful co-ferment action in reactivating ferment extracts from both skeletal and cardiac muscle than adenosinetriphosphoric acid from skeletal muscle. The presence in cardiac tissue of a nucleotide comparable in function to the adenylpyrophosphoric acid of skeletal muscle may be regarded as definitely demonstrated.

AMMONIA PRODUCTION AND AMINO-ACID UTILIZATION. Investigation of the ammonia content of and ammonia production in cardiac muscle is the outcome of the work done on ammonia formation in skeletal muscle. That ammonia appears in muscle and fluids by which muscle or organs have been perfused has been demonstrated by many (Warburg, Posner and Naglein, 1924; Winterstein and Hirschberg, 1925; Embden, 1927; Embden and Schumacher, 1929; Lehnartz, 1929). That activity of skeletal muscle is generally associated with the production of ammonia was first experimentally noted by Embden, Riebeling and Selter (1928) and again by Embden and Schmidt (1930). These workers stated that the ammonia formed was derived from the breakdown of adenylic pyrophosphoric acid. Parnas (1928) stated that the predominant base in muscle from which ammonia could be produced

by deamination was adenine nucleotide and he noted that damage to the muscle caused a rapid production of ammonia. That the purine content of the blood of an animal corresponds to its ability to produce ammonia was put forward by Mozolowski (1928).

Embden and Schmidt (1930) showed that ammonia was produced by enzyme action in frog's and rabbit's muscle by deamination of adenylic acid and not from adenosine, and that the amount of ammonia produced was equal to that obtained by autolysis of untreated muscle preparations in the presence of sodium bicarbonate. That no ammonia can be found in fresh blood (Conway, 1935) and that nitrogen is split off from adenine nucleotide as carbamic acid and not as ammonia (Freund and Lustig, 1931) are opinions opposed by Parnas (1931) and by Parnas and Ostern (1932), who hold that ammonia formation in the surviving frog's heart is not associated with any loss of adenine nucleotide and that the ammonia split off is not utilized for restitution of the nucleotide. Experiments upon the perfused beating heart of the frog have generally shown that ammonia can be recovered in the perfusion fluid. Ostern (1930) in eight experiments on frogs' hearts beating 32 to 38 per minute and kept moist with Ringer's solution found ammonia production vary from 0.0064 to 0.0125 mgm./g./hour. Clark, Gaddie and Stewart (1931) showed that the perfused heart of the frog produces 0.027 mgm. NH_3 nitrogen in 24 hours, the average weight of the heart being 0.196 gram. This means that the frog's heart produces ammonia at the rate of 0.0056 mgm./g./hour. In discussing the question as to the non-utilization of ammonia produced from the breakdown of pyrophosphoric acid (adenyltriphosphoric acid) and the source of the ammonia necessary for restitution of the A. T. P. one must consider the conditions of the experiment. In perfusion by Ringer's solution of a frog's heart suspended in air a certain amount (0.005–0.01 mgm.) of ammonia is lost by diffusion into the atmosphere in 24 hours (Clark *et al.*). This, however, cannot be taken as conclusive proof of the production and subsequent non-reutilization of ammonia; it is proof of a loss of ammonia which may not necessarily take place under normal circulatory conditions. Particularly noteworthy in this connection is the rapidity with which ammonia is produced by damaged cardiac and skeletal muscle. From a normal ammonia content of frog's heart of 1.4 mgm. per cent crushing of the muscle may lead to a production of ammonia which is two-fold in five minutes and may continue to be excessively produced over a period of seven hours (Ostern, 1930). Heart muscle, it would appear is much more active in producing ammonia following cellular damage than is skeletal muscle.

There has been a good deal of controversy over the question of the utilization of ammonia. Parnas (1930) and his fellow-workers maintain that the ammonia produced by the heart is not used in resynthesis of A. T. P. but that resynthesis is brought about by the use of ammonia from amino-acids. This corroborates previous work from Parnas' laboratory (Nachmansohn, 1928; Mozolowski, 1928). Embden (1931) and his school maintain the possibility of a reversal action (Embden, Carstensen and Schumacher, 1928; Embden, Riebeling and Selter, 1928). According to the work of Clark, Gaddie and Stewart (1931) the isolated heart of the frog does utilize amino-acids, giving rise to an increase in ammonia production. Oxygen consumption was not thereby increased. The R.Q. was raised when glycocoll or alanine was added to the perfusion fluid; glycocoll raised the R.Q. from 0.86 to 0.94, alanine raised it from 0.855 to 0.95, leucine lowered the R.Q. from 0.85 to 0.83. Quantitative examination of the figures given in support of protein metabolism of the heart gives less satisfactory results than those quoted. One must bear in mind the minute quantities estimated and the difficulties of accurate oxygen and CO_2 estimation. In the heart-lung preparation of two hours' duration, it would appear that under aerobic conditions the ammonia content of the circulating blood is slightly reduced (Bassani, 1934; Klisiecki, 1934; Cruickshank and McClure, 1936). According to Klisiecki there is, under normal aerobic conditions, a reutilization of ammonia by the heart muscle and the lungs and only in the later hours of perfusion, when anaerobic conditions obtain, is there any production of ammonia by the heart as evidenced by an increase in the blood ammonia.

The question as to whether the isolated heart muscle can make use of ammonia derived from added amino-acids or not depends upon the answer to the question as to whether or not cardiac muscle will deaminate added amino-acids. If the utilization of glycine, alanine, glutamic acid or aspartic acid by muscle would result in the formation of ammonia or urea then sugar formation and its utilization would cause a change in the R.Q. toward unity. It is also sound to conclude that, if the aglycemic heart can show an equicaloric change from carbohydrate to fat, the change from an R.Q. of 0.7 to unity would involve no alteration in caloric requirements. This would mean then that there would be no change in oxygen utilization. If, as Lusk maintains, certain amino-acids upon deamination form glucose, then ammonia formation or amino-acid utilization by the living muscle would be accompanied by an increase in the sugar in the blood or of glycogen in the heart. It has been shown (Cruickshank and Startup, 1933b) that in aglycemia

the R.Q. of the heart falls rapidly from 1.0 to 0.7, the rate of change depending upon whether insulin is given or not. Under such conditions the question arises as to whether or not protein is metabolized in the course of the transformation from carbohydrate to fat oxidation. If an amino-acid such as glycine be utilized by any tissue, sugar, urea and oxygen will be formed; the oxygen formed will not be sufficient for the oxidation of the sugar but in view of the fact that sugar is being burned more CO_2 will be produced. According to Ringer and Lusk 0.5 gram of glycine produces 0.4 gram of carbohydrate which requires approximately 300 cc. of oxygen for its combustion. Since, according to these authors, 75 cc. of oxygen are formed, then 225 cc. of oxygen must be obtained from extraneous sources. Cremer believes that only three-quarters of the carbon of glycine passes over into glucose, which means that 0.5 gram of glycine would form 0.3 gram of glucose and since no oxygen is formed in the breakdown, then for the combustion of the carbohydrate 225 cc. of oxygen are required. The implications attending amino-acid utilization by heart muscle are best seen by considering what would happen if 0.5 gram of glycine were utilized by a 50 gram aglycemic heart utilizing 200 cc. of oxygen and producing 140 cc. of CO_2 per hour. If the aglycemic heart is to revert entirely to glycine for its energy needs it must have available from glycine carbohydrate equivalent to 200 cc. of oxygen, namely, 0.268 gram; that is to say 0.445 gram of glycine must be utilized. Further, this would be associated with an increase of 60 cc. in CO_2 to bring the R.Q. to unity. The demonstration of the utilization of protein or added amino-acids therefore depends upon a record of CO_2 formation, oxygen utilization, sugar formation and combustion, ammonia and urea formation and alterations in the amino-acid nitrogen content of the circulating blood.

The experiments of Cruickshank and McClure (1936) would indicate that there is no change in amino-acid nitrogen or in ammonia or urea formation which would be commensurate with the amount of carbohydrate necessary for a glycine utilization by a heart consuming 200 cc. of oxygen per hour. They conclude that amino-acids are not utilized by the mammalian heart for they emphasize that with a steady oxygen utilization there is a steady fall in CO_2 produced as the sugar rapidly disappears from the blood until the R.Q. reaches 0.7 at which point both O_2 and CO_2 are practically constant. These results corroborate earlier findings that the skeletal muscles in activity do not produce excessive amounts of ammonia and that they are unable to form ammonia from amino-acids (Bollman, Mann and Magath, 1926;

Györgyi and Röthler, 1927; Bornstein and Roese, 1929). Further, they lend support to the finding of Kestner (1932) that of all tissues cardiac muscle contains the least known amount of proteolytic enzymes.

THE ENERGY METABOLISM OF THE HEART. The energy liberated by the heart is utilized to produce heat and to perform work.

Heat production of the heart. Snyder (1926) has shown that the initial heat, that is, the anaerobic moiety of the total heat produced, is for Limulus heart, 26 micro-calories for each centimeter length of muscle per gram tension per beat; for the frog's gastrocnemius and terrapin's ventricle the average figures are 28 and 27 micro-calories per gram weight of muscle per gram tension respectively. The closeness of these figures as well as those for the initial heat tension ratio are regarded as evidence that the fundamental contractile substance is physiologically the same in these three tissues. An analysis of the heat production of the spontaneously beating heart of the terrapin has been carried out by Snyder (1928) which is most clearly summed up in the following table. The figures express absolute calories $\times 10^{-5}$ per gram weight of ventricle muscle per gram tension exerted, per beat.

	INITIAL (SYSTOLIC)	DELAYED (DIASTOLIC)	TOTAL
Anoxidative heat	2.4	0.8	3.2
Oxidative heat	0.1	4.6	4.7
Total anoxidative plus oxidative	2.5	5.4	7.9

These figures which corroborate much previous work (Snyder, 1922 1926–27) show that the heart of the terrapin will beat under anoxybiotic conditions for several hours exerting maximal tensions equal to those produced during oxybiosis. The ratio of the initial and delayed anoxybiotic heats, namely, 1:0.3 is practically that obtained by Hartree and Hill (1922) for similar phases of heat production in sartorius muscle. It is of interest to note that the average total heat produced by the isometrically contracting ventricle of the terrapin, namely, 7.9×10^{-5} calories, is comparable with the figure 7.7×10^{-5} calories obtained for smooth muscle, from the cardiac gastric sphincter of the terrapin (Snyder, 1927) and that the average total initial heat for the cardiac muscle, 2.5×10^{-5} calories compares with the total initial heat of 2.8×10^{-5} calories from the frog's gastrocnemius (Snyder and Gemmill, 1925). These figures for the various phases of heat production are

comparable with those which have been determined by others for skeletal and cardiac muscle contracting isometrically (Gemmill, 1927). Mathematical considerations of the energetics and thermodynamics of contraction have been discussed by Bohnenkamp (1926) and Bohnenkamp and Ernst (1926).

Reference has already been made to the phosphagen index as a criterion of cardiac efficiency and it is not surprising to find attempts being made to demonstrate a relation between the amount of energy released by the heart and its phosphagen content (Clark, 1935). There is, undoubtedly, a relation between oxygen utilization, phosphagen hydrolysis and lactic acid formation. Under conditions of asphyxiation of the iodo-acetic acid poisoned frog's ventricle, there is a definite loss of phosphagen from the ventricle and it has been suggested that this loss corresponds with the energy expenditure of the heart. The heat equivalent of lactic acid formation in cardiac muscle and neutralization may vary from 230 to 380 calories per gram depending upon the degree to which inorganic salts and protein may be used to neutralize the acid. The normal production of 5 mgm. lactic acid per gram per hour by the frog's ventricle corresponds to a heat liberation of from 1.15 to 1.9 calories per gram per hour. If the energy liberated per beat during asphyxia is the same in the iodo-acetic acid poisoned as in the normally beating ventricle then the amount of heat produced per gram per hour may be related to the amount of phosphagen hydrolyzed. A heat liberation of 1.15 to 1.90 calories per gram per hour is equivalent to the liberation of 3.24 to 5.35 mgm. phosphagen P which would give, with a heart beating at the rate of 15 per minute, 3.60 to 5.94 γ phosphagen P per gram per beat. If then the normal rate of lactic acid production by the heart be 5 mgm. per gram per hour, there would be a production of 5.55 γ lactic acid per gram per beat, corresponding to an average loss of 4.77 γ of phosphagen P. The phosphagen content of the normal frog's heart is approximately 7.0 mgm. per cent, that is, 10 γ of phosphagen P per gram of muscle. From such calculations one would conclude that the iodo-acetic acid poisoned and asphyxiated ventricle has a very short period of survival. Such calculations can scarcely be taken as indicating any close or even approximate correspondence between the amount of phosphagen used and the energy released in the iodo-acetic acid poisoned ventricle contracting anaerobically, or that creatine phosphate is the only or chief source of energy production in cardiac muscle.

Cardiac efficiency. The efficiency of the contraction is determined

by the ratio of the thermal equivalent of the work done and the calories of heat produced. This can be determined indirectly by comparing the total oxygen utilized with the amount of work which has been performed. It is, however, extremely difficult to evaluate all the factors involved in the production of work by the heart. Many attempts have been made to assess correctly the static and kinetic factors and various formulae have been proposed (Rhode, 1912; Evans and Matsuoka, 1915; Evans, 1918; Katz, 1932). A full account of the work done on this aspect of the problem with references to the various formulae will be found in the paper by Wiggers and Katz (1928). Starling (1915) showed that the normal heart dilates when increased work is imposed upon it and that the pathological heart dilates without any increased demands being thrown upon it. The physiological significance of this is that the magnitude of energy liberated upon cardiac contraction is a function of the length of the muscle fibres of the ventricles at the end of diastole. If this be so, then the oxygen consumption of the heart should be determined by the initial length of the cardiac fibre and for any one heart the oxygen consumption should remain constant for any determined fibre length. In a normal heart the diastolic volume should be constant for any given amount of work. These are the conditions upon which depend the law of the heart as put forward by Starling (1915). There are, however, limits within which the law operates; these are determined by the filling or venous pressure, the emptying or arterial pressure, the rate of contraction, which controls the time of filling and the temperature, which determines the rate of relaxation, the energy liberated in and the duration of the contraction period (Starling and Visscher, 1927; Moldavsky and Visscher, 1933, and Scheinfinkel, 1931). It has been shown that below a critical contraction rate and above a critical filling pressure the diastolic volume of the ventricle is independent of the arterial pressure. This is best elaborated by quoting Moldavsky and Visscher (1933). "Whether or not there will be an increase in diastolic volume with increased arterial pressure depends upon the point on the filling curve after systole that a new contraction begins. If the filling curve has reached its plateau, as occurs at the lower heart rates, the higher venous pressures and the higher temperatures, the arterial pressure is without effect upon the diastolic volume because the extent of emptying has no influence upon the level of filling reached in diastole." It is clear therefore that the "law of the heart" with respect to adaptation to increased pressure load must be modified to include the factors

enumerated. Stella (1931) and Peserico (1928) put forward evidence for believing that with a *constant* diastolic pressure, an increase in arterial pressure led to increased liberation of energy by the heart. While Stella's conclusions are not generally accepted, his work showed that the ratio, (increase of anaerobic energy)/(increase of work done), of 1.16 is in agreement with the figures of Hill (1930) which show for the frog's sartorius contracting isotonically against increasing loads, a close parallelism between work and anaerobic energy.

It is known that the tension-time value bears a constant relation to heat production in skeletal muscle (Hartree and Hill, 1921) and that it is a good index of mechanical activity. It is also known that the after loaded muscle when it contracts develops additional energy to that produced when it contracts isometrically (Fenn, 1923–24). In a valuable paper on the static and dynamic effort of the heart during its ejection phase, Wiggers and Katz (1928) have made use of a method whereby they evaluate the mechanical energy of the heart by taking into account the changing factor of capacity when examining the tension-time values of the after loaded ventricular contraction. They have shown that with constant heart rate, increase in initial length of the ventricle fibre, which is not excessive and is not accompanied by any increase in arterial pressure, leads to a more efficient distribution of the energy in that a greater proportion is available for dynamic purposes, which may amount to a 30 to 50 per cent increase above normal dynamic needs. When initial length remains unchanged, the heart rate again being constant and arterial pressure is varied, the velocity of ejection of blood is decreased and the kinetic energy is therefore diminished. The higher diastolic pressure which results requires a greater proportion of the energy to be utilized in the static effort of overcoming and maintaining the greater arterial resistance. This means that there is an 18 to 35 per cent decrease in the amount of energy available for dynamic purposes. The initial length of the ventricular muscle therefore governs the systolic discharge and regulates the economy with which the pressure energy is utilized. There is thus present a means whereby cardiac energy may be conserved not only when the heart is required to increase its systolic output as a result of increased venous inflow, but also when changes in rate or arterial resistance demands an increase in the work to be done. Diastolic rest obtained with a slow heart is not the only factor in maintaining physiological activity; more important in the development of energy is the change in initial length which prolonged diastolic filling secures. In-

creased arterial tension *per se* has been shown to be without any stimulating effect on the ventricle (Wiggers and Katz). It is important that as large a proportion as possible of the developed energy should be available for dynamic purposes. The physiological mechanism for the conservation of energy in the heart facing stresses and strains is one which is responsible for the maintenance of a normal relation between energy available for static and kinetic or dynamic purposes. In the failing heart the defect is an inability to employ energy for useful and necessary work; it is not entirely an inability to liberate the normal amount of energy. The failing heart has been found to liberate energy in contraction to the same extent at any fibre length as the normal heart muscle (Dechard and Visscher, 1933). The implications of such findings for clinical medicine are apparent. It is not a question of fuel but of repair material, it is not a question of determining what increases energy production but what conserves energy or maintains tonus or efficiency, or in other words, what restores the normal balance between static and dynamic requirements. For example, adrenaline will cause a great increase in energy output, but a marked lowering of efficiency (Starling and Visscher), while insulin will restore efficiency without any definite increase in energy liberation (Visscher and Müller, 1927; Bodo, 1928).

Oxygen consumption in relation to initial length. Feng (1932) showed that oxygen consumption and heat production of resting sartorius muscle increased with increase in the length of the muscle fibre, and Evans and Hill (1914) had long before shown that heat production in the tetanized sartorius muscle increased as the muscle was extended. Similar results on the isolated heart were obtained by Starling and Visscher, who showed increased oxygen utilization with increase in diastolic volume. Smith (1934) has demonstrated that the oxygen consumption of active sartorius muscle rises to a maximum when the length is increased by 24 per cent of the normal and declines upon further increase to 36.5 per cent of normal or minimal length. Such data would indicate that "the response of both cardiac and skeletal muscle to increase in fibre length is identical, since that portion of the metabolism not directly associated with recovery from activity becomes an increasingly significant part of total metabolism at appreciably increased initial fibre length."

REFERENCES

ANDERSON, I. A., R. A. CLEGHORN, J. J. R. MACLEOD AND J. M. PETERSON. J. Physiol. **71**: 391, 1931.

ANDERSON, R. J. AND G. LUSK. J. biol. Chem. **32**: 421, 1917.
ARNING, D. J. Physiol. **63**: 107, 1927.
BACKMANN, H. Pflüger's Arch. **217**: 151, 1927.
BARCROFT, J. AND W. E. DIXON. J. Physiol. **35**: 182, 1907.
BASSANI, B. Arch. di Fisiol. **33**: 390, 1934.
BAYLISS, L. E., A. R. FEE AND E. OGDEN. J. Physiol. **66**: 443, 1928.
BAYLISS, L. E., E. A. MÜLLER AND E. H. STARLING. J. Physiol. **65**: 33, 1928.
BEATTIE, F., T. H. MILROY AND R. W. M. STRAIN. Biochem. J. **28**: 84, 1934.
BENEDICT, S. R. Am. J. Physiol. **13**: 192, 1905.
BENEDICT, F. G. AND E. P. CATHCART. Publications Carnegie Inst., no. 181, 1913.
BENNET, D. W. AND A. N. DRURY. J. Physiol. **72**: 288, 1931.
BISSINGER, E., E. J. LESSER AND K. ZIPF. Klin. Wchnschr. **2**: 2233, 1923.
BLUME, H. Beitr. z. Path. u. z. allg. Path. **93**: 20, 1934.
BODENHEIMER, W. Arch. exper. Path. Pharmakol. **80**: 77, 1916.
BODO, R. J. Physiol. **64**: 365, 1928.
BOGUE, J. Y., C. L. EVANS AND F. Y. HSY. Quart. J. exper. Physiol. **25**: 213, 1935.
BOHNENKAMP, H. Ztschr. f. Biol. **84**: 79, 1926.
BOHNENKAMP, H. AND W. ERNST. Ztschr. f. Biol. **84**: 436, 1926.
BOHNENKAMP, H., G. EISMAYER AND Z. ERNST. Ztschr. f. Biol. **87**: 489, 1928.
BOHNENKAMP, H. AND W. ERNST. Münch. Med. Wchnschr. **74**: 174, 1927.
BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH. Am. J. Physiol. **78**: 258, 1926.
BONG, E., P. JUNKERSDORF AND H. STEINBORN. Ztschr. f. d. ges. exper. Med. **92**: 573, 1934.
BORNSTEIN, F. Biochem. Ztschr. **209**: 172, 1929.
BORNSTEIN, A. AND H. F. ROESE. Biochem. Ztschr. **212**: 127, 1929.
BOYLAND, E. Biochem. J. **22**: 362, 1928.
BRODY, H. Am. J. Physiol. **93**: 190, 1930.
BURN, J. H. AND H. H. DALE. J. Physiol. **59**: 164, 1924.
CAMIS, M. Arch. Ital. de Biol. **91**: 35, 1934.
CHEN, K. K. Personal communication, 1934.
CHEVALIER, J. AND A. BRISSEMORET. Compt. Rend. Acad. Sci. Paris **147**: 217, 1908.
CLARK, A. J. Quart. J. Exper. Physiol. **25**: 167, 1935.
CLARK, A. J., G. P. EGGLETON AND P. EGGLETON. J. Physiol. **72**: 25, 1931.
J. Physiol. **75**: 332, 1932.
CLARK, A. J., R. GADDIE AND C. P. STEWART. J. Physiol. **70**: 7, 1930.
J. Physiol. **72**: 443, 1931.
J. Physiol. **75**: 311, 1932.
J. Physiol. **82**: 265, 1934.
CLARK, A. J., G. H. PERCIVAL AND C. P. STEWART. J. Physiol. **66**: 346, 1928.
CLARK, A. J. AND A. C. WHITE. J. Physiol. **66**: 185, 1928.
J. Physiol. **68**: 406, 1929–30.
CONWAY, E. J. Biochem. J. **29**: 2755, 1935.
CORI, C. F. AND G. T. CORI. J. Biol. Chem. **70**: 557, 1926.
J. Biol. Chem. **79**: 321, 1928.
CORI, C. F., G. T. CORI AND G. W. PUCHER. J. Pharmacol. **21**: 377, 1923.
CORKHILL, A. B., H. H. DALE AND H. P. MARKS. J. Physiol. **70**: 86, 1930.

CRUICKSHANK, E. W. H. J. Physiol. **47:** 1, 1913.
J. Physiol. **82:** 26, 1934.
J. Physiol. **81:** P. 45, 1934.
CRUICKSHANK, E. W. H. AND G. S. MCCLURE. J. Physiol. **86:** 1, 1936.
CRUICKSHANK, E. W. H., G. S. MCCLURE AND D. L. MACKINTOSH. Report to Banting Research Foundation, Toronto, 1935.
CRUICKSHANK, E. W. H. AND D. L. SHRIVASTAVA. Am. J. Physiol. **92:** 144, 1930.
CRUICKSHANK, E. W. H. AND C. W. STARTUP. J. Physiol. **70:** P. 5, 1930.
J. Physiol. **77:** 365, 1933.
J. Physiol. **80:** 179, 1933b.
J. Physiol. **81:** 153, 1934.
DALE, H. H. Lancet **204:** 989, 1923.
DECHARD, G. AND M. B. VISSCHER. Am. J. Physiol. **103:** 400, 1933.
J. Exper. Med. **59:** 195, 1933.
DICKENS, F. Nature **131:** 130, 1933.
DRURY, A. N. AND E. C. ANDRUS. Heart **11:** 389, 1924.
DRURY, A. N. AND A. SZENT-GYÖRGYI. J. Physiol. **68:** 213, 1929–30.
DUDLEY, H. W. Biochem. J. **25:** 439, 1931.
EGGLETON, G. P. AND P. EGGLETON. J. Physiol. **68:** 193, 1929.
EGGLETON, M. G. AND C. L. EVANS. J. Physiol. **70:** 261, 1930.
EISMAYER, G. AND H. QUINCKE. Klin. Wchnschr. **8:** 1853, 1929.
Ztschr. f. Biol. **88:** 139, 1929.
Ztschr. f. Biol. **89:** 523, 1930.
EMBDEN, G. Klin. Wchnschr. **6:** 628, 1927.
Arch. Exp. Path. Pharmakol. **167:** 50, 1932.
Ztschr. physiol. Chem. **196:** 23, 1931.
EMBDEN, G., M. CARSTENSEN AND H. SCHUMACHER. Ztschr. physiol. Chem. **179:** 186, 1928.
EMBDEN, G. AND H. J. DEUTICKE. Ztschr. physiol. Chem. **230:** 50, 1934.
EMBDEN, G., C. RIEBELING AND G. E. SELTER. Ztschr. physiol. Chem. **179:** 149, 1928.
EMBDEN, G. AND H. SCHUMACHER. Pflüger's Arch. **223:** 487, 1929.
EMBDEN, G. AND G. SCHMIDT. Ztschr. physiol. Chem. **186:** 205, 1930.
Ztschr. physiol. Chem. **181:** 130, 1929.
EMBDEN, G. AND M. ZIMMERMANN. Ztschr. physiol. Chem. **167:** 137, 1927.
EVANS, C. L. J. Physiol. **45:** 213, 1912–13.
J. Physiol. **47:** 407, 1913–14.
J. Physiol. **52:** 6, 1918–19.
EVANS, C. L., A. C. DE GRAFF, T. KOSAKA, K. MACKENZIE, E. MURPHY, T. VACEK, D. H. WILLIAMS AND F. G. YOUNG. J. Physiol. **80:** 21, 1933.
EVANS, C. L., F. GRANDE AND F. Y. HSU. Quart. J. Exper. Physiol. **24:** 283, 1934.
Quart. J. Exper. Physiol. **24:** 347, 1935.
EVANS, C. L., F. GRANDE, F. Y. HSU, D. H. K. LEE AND A. G. MULDER. Quart. J. Exper. Physiol. **24:** 365, 1935.
EVANS, C. L. AND A. V. HILL. J. Physiol. **49:** 10, 1914.
EVANS, C. L., F. Y. HSU AND T. KOSAKA. J. Physiol. **82:** 41, 1934.
EVANS, G. J. Physiol. **82:** 468, 1934.

FENG, T. P. J. Physiol. **74**: 441, 1932.
FENN, W. O. J. Physiol. **58**: 175, 1923–24.
FERGUSON, J. K., L. IRVING AND F. B. PLEWES. J. Physiol. **69**: 113, 1930.
FISCHER, E. Pflüger's Arch. **216**: 123, 1927.
FISHER, N. F. AND R. W. LACKEY. Am. J. Physiol. **72**: 43, 1925.
FISKE, C. H. AND Y. SUBBAROW. J. Biol. Chem. **66**: 375, 1925.
Science **70**: 382, 1929.
FITZ, R., W. P. MURPHY AND G. B. GRANT. J. Metabol. Res. **2**: 753, 1922.
FLETCHER, W. M. AND F. G. HOPKINS. J. Physiol. **35**: 247, 1907.
FREUND, H. AND W. KÖNIG. Arch. exper. Path. u. Pharmakol. **129**: 193, 1927.
FREUND, E. AND B. LUSTIG. Biochem. Ztschr. **232**: 442, 1931.
FUKUDA AND NAITO. Proc. Japan. Pharmacol. Soc. **1**: 40, 1927.
FURUSAWA, K. AND P. M. T. KERRIDGE. J. Physiol. **63**: 33, 1927.
GADDIE, R. AND C. P. STEWART. J. Physiol. **80**: 457, 1934.
GAULE. Arch. f. Physiol. 1878 (cited from E. G. MARTIN).
GEIGER, E. AND E. SCHMIDT. Arch. f. exper. Path. u. Pharmakol. **134**: 173, 1928.
GEMMILL, C. L. Am. J. Physiol. **80**: 668, 1927.
Am. J. Physiol. **83**: 415, 1928.
Am. J. Physiol. **108**: 55, 1934.
GOTTDENKER, F. AND M. WACHSTEIN. Biochem. Ztschr. **267**: 192, 1933.
GOTTLIEB, E. Biochem. Ztschr. **194**: 163, 1928.
GOTTSCHALK, A. Arch. exper. Path. u. Pharmakol. **75**: 33, 1913.
GYÖRGYI, P. AND H. RÖTHLER. Biochem. Ztschr. **187**: 194, 1927.
HAENDEL, M. AND A. MUNILLA. Biochem. Ztschr. **212**: 35, 1929.
HARTREE, W. AND A. V. HILL. J. Physiol. **55**: 133, 1921.
J. Physiol. **56**: 367, 1922.
HEMINGWAY, A. AND H. J. PHELPS. J. Physiol. **80**: 369, 1934.
HEPBURN, J. AND J. K. LATCHFORD. Am. J. Physiol. **62**: 177, 1922.
HEWITT, J. A. AND D. DE SOUZA. J. Physiol. **54**: p. 119, 1921.
HILL, A. V. J. Physiol. **56**: 367, 1922.
Proc. Roy. Soc. B. **107**: 115, 1930.
Physiol. Rev. **12**: 56, 1932.
HILL, A. V., C. N. H. LONG AND H. LUPTON. Proc. Roy. Soc. B. **96**: 455, 1924.
HIMWICH, H. E. AND W. B. CASTLE. Am. J. Physiol. **83**: 92, 1927.
HIMWICH, H. E. AND M. I. ROSE. Am. J. Physiol. **88**: 663, 1929.
Am. J. Physiol. **6**: 193, 1901.
HOWELL, W. H. Am. J. Physiol. **6**: 293, 1901.
IRVING, J. T. Biochem. J. **20**: 613, 1926.
JOWETT, M. AND J. H. QUASTEL. Biochem. J. **27**: 487, 1933.
JUNKERSDORF, P. Pflüger's Arch. **210**: 351, 1925.
JUNKERSDORF, P. AND P. TÖRÖK. Pflüger's Arch. **211**: 414, 1926.
JUNKERSDORF, P. AND S. HANISCH. Arch. exper. Path. u. Pharmakol. **123**: 243, 1927.
KATZ, L. N. Am. J. Physiol. **99**: 579, 1932.
KESTNER, O. XIV International Physiological Congress, Rome, 1932.
KILBORN, L. G. J. Physiol. **66**: 403, 1928.
KILBORN, L. G., S. SOSKIN AND J. C. THOMAS. Trans. Roy. Soc. Can., Sect. 5, 1928.
KLISIECKI, A. J. Quart. J. Exper. Physiol. **24**: 225, 1934.

KNOWLTON, F. P. AND E. H. STARLING. J. Physiol. **44:** 206, 1912.
J. Physiol. **45:** 146, 1912.
KOSTERLITZ, H. Arch. f. exper. Path. u. Pharmakol. **173:** 159, 1933.
KREBS, H. A. Ztschr. physiol. Chem. **217:** 177, 1933.
KREMER, M., A. J. WILSON AND S. WRIGHT. J. Physiol. **82:** 414, 1934.
KROGH, A. The respiratory exchange in animals and man. 1916.
Deutsch. Med. Wchnschr. **40:** 1321, 1923.
KROGH, A. AND J. LINDHARD. Biochem. J. **14:** 290, 1920.
KULZ, E. Festschr. zu Ludwig, Marburg, 1891, p. 109, (cited from LUSK).
LAFON, G. Recherches expérimentales sur la diabète et sur la glycogénie, cited from RAPPORT, Physiol. Rev. **10:** 419, 1930.
LANGENDORF, O. Pflüger's Arch. **61:** 291, 1895.
LAWRENCE, R. D. AND R. A. MCCANCE. Biochem. J. **25:** 570, 1931.
LEATHES, J. B. Problems in animal metabolism. London, 1906.
LEHNARTZ, M. Ztschr. physiol. Chem. **184:** 183, 1929.
LINGLE, D. J. Am. J. Physiol. **8:** 98, 1902.
Am. J. Physiol. **14:** 433, 1905.
LOCKE, F. S. AND O. ROSENHEIM. J. Physiol. **36:** 205, 1907.
LOEPER, M. AND A. LEMAIRE. Compt. rend. Soc. de Biol. **103:** 209, 1930.
LOHMANN, K. Naturwissenschaft. **17:** 624, 1929.
Naturwissenschaft. **19:** 180, 1931.
Biochem. Ztschr. **233:** 460, 1931.
Biochem. Ztschr. **254:** 332, 1932.
Biochem. Ztschr. **271:** 264, 1934.
LOHMANN, K. Ztschr. angew. Chem. **48:** 165, 1935.
LOHMANN, K. AND O. MEYERHOF. Biochem. Ztschr. **273:** 60, 1934.
LUNDSGAARD, E. Biochem. Ztschr. **217:** 162, 1930.
Biochem. Ztschr. **227:** 51, 1930.
LUSCHER, E. Ztschr. f. Biol. **72:** 109, 1920.
Ztschr. f. Biol. **72:** 107, 1920.
Ztschr. f. Biol. **73:** 67, 1921.
Ztschr. f. Biol. **84:** 297, 1926.
MCGINTY, D. A. AND A. T. MILLER. Am. J. Physiol. **103:** 712, 1933.
MACLEAN, H. AND I. SMEDLEY. J. Physiol. **45:** 462, 470, 1913.
MACLEOD, J. J. R. Physiol. Rev. **4:** 21, 1924.
Carbohydrate metabolism and insulin. Longmans, Green & Co., London, 1926.
The fuel of life. Oxford Univ. Press, 1928.
MACLEOD, J. J. R. AND D. S. PRENDERGAST. Trans. Roy. Soc. Canada 1921, Sec. V, 37.
MACPHERSON, W. E., H. E. ESSEX AND F. C. MANN. Am. J. Physiol. **99:** 429, 1931–32.
MANSFELD, G. M. AND K. H. HECHT. Pflüger's Arch. **232:** 666, 1933.
MARTIN, E. G. Am. J. Physiol. **15:** 303, 1905–06.
MERUNOVICZ. Arbeiten an der physiol Anstalt zu Leipzig, p. 132, 1875.
MEYERHOF, O. Pflüger's Arch. **185:** 11, 1920.
Pflüger's Arch. **188:** 114, 1921.
Die Chemischen Vorgánge in Muskel. Berlin, Julius Springer, 1930.
Nature **132:** 337, 373, 1933.

MEYERHOF, O. AND E. BOYLAND. Biochem. Ztschr. **237**: 406, 1931.
MEYERHOF, O., K. LOHMANN AND R. MEIER. Biochem. Ztschr. **157**: 459, 1925.
MILROY, T. H. Physiol. Rev. **11**: 515, 1931.
MINKOWSKI, O. (1924) cited from HAWLEY AND MURLIN, Am. J. Physiol. **75**: 107, 1925–26.
MOLDAVSKY, L. F. AND M. B. VISSCHER. Am. J. Physiol. **106**: 329, 1933.
MOWAT, D. M. AND C. P. STEWART. Biochem. J. **28**: 774, 1934.
MOZOLOWSKI, W. Klin. Wchnschr. **206**: 150, 1928.
MÜLLER, J. Ztschr. allg. Physiol. **3**: 282, 1904.
MURLIN, J. R., L. EDELMANN AND B. KRAMER. J. Biol. Chem. **16**: 79, 1913.
NACHMANSOHN, D. Biochem. Ztschr. **196**: 73, 1928.
NAGAYA, T. Pflüger's Arch. **221**: 733, 1929.
NEEDHAM, J. Quart. J. Exper. Physiol. **18**: 153, 1927.
Biochem. J. **17**: 422, 1923.
NEEDHAM, D. M. Biochemistry of muscle. London, Methuen & Co., 1932.
NEEDHAM, D. M. AND W. E. V. HEYNINGEN. Biochem. J. **29**: 2040, 1935.
OSTERN, P. Biochem. Ztschr. **228**: 401, 1930.
Biochem. Ztschr. **270**: 1, 1934.
PACE, L. Arch. Sci. Biol. **17**: 447, 1932.
PALAZZOLO, G. Arch. Fisiol. **11**: 558, 1912.
PARNAS, J. K. Klin. Wchnschr. **7**: 1423, 1928.
Biochem. Ztschr. **239**: 18, 1931.
Ann. Rev. Biochem. **1**: 431, 1932.
Ann. Rev. Biochem. **2**: 117, 1933.
PARNAS, J. K., W. LEWINSKI, J. JAWORSKA AND B. UMSCHWEIF. Biochem. Ztschr. **228**: 366, 1930.
PARNAS, J. K. AND P. OSTERN. Biochem. Ztschr. **248**: 392, 1932.
Biochem. Ztschr. **279**: 94, 1935.
PARNAS, J. K., P. OSTERN AND T. MANN. Biochem. Ztschr. **272**: 64, 1934.
Biochem. Ztschr. **275**: 74, 1935.
Biochem. Ztschr. **275**: 163, 1935.
PATTERSON, S. W. AND E. H. STARLING. J. Physiol. **47**: 137, 1913.
PESERICO, E. Arch. di Fisiol. **23**: 488, 1926.
J. Physiol. **65**: 146, 1928.
PETERSON, J. M. J. Physiol. **79**: 508, 1933.
PLATTNER, F. J. Physiol. **59**: 289, 1924.
POHLE, K. Ztschr. physiol. Chem. **184**: 261, 1929.
RAPPORT, D. Am. J. Physiol. **91**: 238, 1929.
RAPPORT, D. AND E. D. RALLI. Am. J. Physiol. **83**: 450, 1928.
REDFIELD, A. C. AND D. N. MEDEARIS. Am. J. Physiol. **77**: 662, 1926.
REID, C. AND B. NARAYANA. Biochem. J. **25**: 339, 1931.
RHODE, E. Ztschr. physiol. Chem. **68**: 181, 1910.
Arch. exper. Path. u. Pharmakol. **68**: 401, 1912.
ROTHSCHILD, P. Biochem. Ztschr. **217**: 365, 1930.
RÜHL, A. Klin. Wchnschr. **13**: 1529, 1934.
SACHS, F. Pflüger's Arch. **115**: 550, 1906.
SAHYUN, M. J. Biol. Chem. **93**: 227, 1931.
SCHEINFINKEL, N. Ztschr. Biol. **84**: 297, 1926.
Ztschr. f. Biol. **91**: 322, 1931.

SCHMIDT, A. Ztschr. physiol. Chem. **179:** 243, 1928.
SMITH, P. W. Proc. Soc. exper. Biol. and Med. **32:** 257, 1934.
SNYDER, C. D. Am. J. Physiol. **59:** 254, 1922.
Am. J. Physiol. **76:** 170, 1926.
Am. J. Physiol. **79:** 719, 1927.
Am. J. Physiol. **84:** 69, 1928.
SNYDER, C. D. AND C. L. GEMMILL. Am. J. Physiol. **73:** 556, 1925.
SOSKIN, S. AND L. N. KATZ. XIV International Congress, Rome, 1932.
SPRAGUE, P. H. Am. J. Physiol. **105:** 402, 1933.
STARLING, E. H. Linacre Lecture on the law of the heart. 1915. London, Longmans Green & Co., 1918.
STARLING, E. H. AND C. L. EVANS. J. Physiol. **49:** 67, 1914–15.
STARLING, E. H. AND M. B. VISSCHER. J. Physiol. **62:** 243, 1926–27.
STEINBERG, S. J. Pflüger's Arch. **217:** 694, 1927.
STELLA, G. J. Physiol. **72:** 247, 1931.
STRAIN, R. W. M. Biochem. J. **28:** 90, 1934.
TAKANE, R. Biochem. Ztschr. **171:** 403, 1926.
TANZI, B. Ateneo parmense **5:** 549, 1933.
Ateneo parmense **5:** 575, 1934.
UNDERHILL, F. P. AND A. L. PRINCE. J. Biol. Chem. **17:** 299, 1914.
VERZÁR, F. Biochem. Ztschr. **39:** 52, 1911.
VISSCHER, M. B. AND A. G. MULDER. Am. J. Physiol. **94:** 630, 1930.
VISSCHER, M. B. AND E. A. MÜLLER. J. Physiol. **62:** 341, 1927.
VOEGTLIN, C., S. M. ROSENTHAL AND J. M. JOHNSON. Pub. Health Repts. **46:** 339, 1931.
WARBURG, O., K. POSNER AND E. NAGLEIN. Biochem. Ztschr. **152:** 309, 1924.
WEIZSACKER, V. Pflüger's Arch. **141:** 457, 1911.
Pflüger's Arch. **147:** 135, 1912.
Pflüger's Arch. **148:** 535, 1912.
WERTHEIMER, E. Pflüger's Arch. **225:** 429, 1930.
Pflüger's Arch. **225:** 499, 1930.
Pflüger's Arch. **227:** 100, 1931.
Pflüger's Arch. **232:** 120, 1933.
WIGGERS, C. J. AND L. N. KATZ. Am. J. Physiol. **85:** 229, 1928.
WINFIELD, G. J. Physiol. **49:** 171, 1915.
WINTERSTEIN, H. AND E. HIRSCHBERG. Biochem. Ztschr. **156:** 138, 1925.
WITTING, V., J. MARKOWITZ AND F. C. MANN. Am. J. Physiol. **94:** 35, 1930.
YATER, W. M., A. E. OSTERBERG AND H. W. HEFKE. Proc. Staff, Mayo Clinic **3:** 243, 1928.
YATER, W. M., T. MARKOWITZ AND R. F. CAHOON. Arch. int. Med. **51:** 800, 1933.
ZUNT, N. Oppenheimer's Handbuch. Biochem., Jena **4:** 857, 1911.

THE BLOOD CALCIUM AND THE CALCIUM FACTOR IN BLOOD COAGULATION

JOHN H. FERGUSON

Department of Physiology and Pharmacology, University of Alabama School of Medicine

The physiological chemistry of the blood calcium. The extensive modern literature on the rôle of calcium in the body is treated at length in earlier numbers of these reviews by Stewart and Percival (1928), Thompson and Collip (1932), and Schmidt and Greenberg (1935), but scant attention is given to the important functions of calcium in blood coagulation. For a discussion of this topic it is necessary, briefly, but critically, to summarise the salient modern conclusions concerning the blood calcium.

Distribution of calcium in the blood. Technical difficulties and various sources of error and misinterpretation have contributed to considerable confusion and hampered the reaching of a final verdict regarding the exact distribution of calcium in the blood. Apart from a general consensus of opinion among modern investigators that the data obtained prior to 1917 lack the technical accomplishment to rank with the present day methods of oxalation and $KMnO_4$ titration, overt suspicion attaches to certain techniques, such as that of Lyman, which have enjoyed periods of popular usage. It may still be queried whether the estimation of calcium in whole blood has reached the precision of serum methods, and the query is pertinent, inter alia, to the important comparisons by which it is sought to answer the question of the calcium content of the blood corpuscles.

Whole blood. Attention is called to *a*, difficulties in attaining a reliable technique, and *b*, the necessity for including hematocrit values with all mineral analyses of whole blood. A representative modern series (147) averages 6 mgm. of Ca for 100 cc. of human whole blood, with a mean plasma volume of 60 per cent.

Cells. Abderhalden (1) could find no calcium in the direct analysis of washed blood corpuscles. Kramer and Tisdall (86) reviewed the older literature and Schmidt and Greenberg (155) the modern data in support of the current conclusion that the interior of the corpuscles contain

less than 0.5 mgm. per cent of calcium. A number of discrepancies are cited by Anderson (3), v. Gulácsy (53), and Schmidt and Greenberg (155).

Plasma. There has been a general tendency to ignore differences (averaging 1 mgm. per cent, 130) between plasma and serum calcium values, although the lack of exact correspondence has been reiterated. Native (cooled) plasma, obtained in non-wettable vessels, without the use of anticoagulants (cf. 185), was found by Stewart and Percival (169) to yield 10 to 20 per cent higher calcium values than the corresponding citrated plasma or serum. Cameron and Moorhouse (22) noted *a*, plasma dilution owing to osmotic effects of the anticoagulant, and *b*, a slight precipitation of calcium (as phosphate) owing to disturbances of the interionic equilibria, as factors tending to lower the calcium values for citrated plasma. Hematocrit values (86, 53, 156, 63) would appear able to afford the slight correction necessary; or isotonic (3.8 per cent, 66) sodium citrate ($\frac{1}{10}$ volume) could be used, with suitable recalculation of volume. Heparinised plasma has been used occasionally (23, 103, 185, 82) but insufficient cognizance seems to have been taken of the fact that heparin contains appreciable amounts of calcium (1.7 mgm., Holt, 67; 2.1 mgm. per cent, Waelsch et al., 185). Heiduschka and Schmidt-Hebbel (62) investigated the usefulness of a variety of anticoagulants in the preservation of plasma for calcium determinations. Calcium precipitants such as fluoride and oxalate (cf. Rothwell, 146) were quite unsuitable. Sodium citrate ($\frac{1}{10}$ volume of 5 per cent solution) required the addition of a correction factor of about 1 mgm. per cent. Hirudin, Liquoid, and Germanin gave plasmas which yielded calcium values closely approximating those of serum. Liquoid, Germanin, and Novirudin, as well as citrates, were stated (125) to "react with calcium ions in aqueous solutions to form complex ions."

The foregoing data suggest that values for plasma calcium should be regarded critically, notwithstanding their tendency to approximate to the serum figures in the hands of a number of the most reliable investigators. The final word on the ability of calcium to diffuse into corpuscles may not have been said (Guillaumin, 1930; Waelsch, Kittel, Busztin, 1935), but the quoted results of analyses on washed cells, coupled with comparisons of whole blood values *a*, directly determined, and *b*, recalculated from plasma (or serum), are generally considered to demonstrate the absence of *combined* calcium in the blood cells.

Serum. There is abundant evidence for the clinical reliability of

routine calcium determinations made on blood serum. Schmidt and Greenberg (1935) cited the best current analyses of the calcium fractions (and several other constituents) of the sera of man and certain additional species. Human serum calcium totals between 9.0 and 11.5 mgm. per cent in different individuals. In the same individual, considerably smaller variations than this are encountered under most physiological and pathological conditions (with a few important exceptions). The dog, rat, guinea pig, and ox show similar values to the human, but the rabbit, pig and hen (especially when laying) commonly yield higher figures.

Physical state of serum calcium. There has accumulated considerable evidence to confirm and extend Rona and Takahashi's (143–144) original observation that the serum calcium exists in at least two forms, termed diffusible and non-diffusible (combined). Practical methods of separation and estimation (discussed in the reviews cited) include *a*, compensation dialysis; *b*, vividiffusion, and *c*, ultrafiltration. Schmidt and Greenberg (155) argued for an analogy of principle as between diffusion and filtration methods, and also dealt with the vexed question of the rôle of Donnan membrane effects. Greenberg's (155) ultrafiltration data on *dog* sera agreed closely with Greene and Power's (50) vividiffusion results, both series yielding an average of 62 per cent of diffusible: total calcium. With ordinary collodion membranes at low pressures the filtration figures cited for *human* sera averaged 53 per cent. In addition to temperature and type of membrane, variables connected with pH and with filtration pressure require control.

A. NATURE OF NON-DIFFUSIBLE CALCIUM. *Proteinates.* Since the work of Rona and Takahashi (143–144), it has been widely held that the non-diffusible calcium exists in combination with the serum proteins. Loeb (98), Loeb and Nichols (99–101), and Marrack and Thacker (111) experimented with serum and serum proteins separated from calcium salt solutions by a collodion dialysing membrane. At equilibrium (pH 7.4) the partition of calcium could be accounted for by *a*, Donnan membrane effects plus *b*, a considerable factor believed to be due to the formation of an undissociated calcium-protein compound. The primary dependence upon protein and total calcium content is modified by secondary variables such as pH and the other salts (especially NaCl) present. Significant analogies for the assumption of the calcium-protein complex include *a*, Northrop and Kunitz' (127, 128) E.M.F. data on gelatine solutions containing zinc and other salts, and *b*, observations of Schmidt and his co-workers (cited 155) on the

electrical migration (cataphoresis) of the alkaline earth caseinates. We have been unable to find published data on the corresponding phenomena with serum calcium proteinates.

That the non-diffusible calcium is fairly firmly bound is evident from the difficulty experienced in mobilising it by simple dialysis. The above workers (98–101, 111), however, were able to free serum proteins from all calcium, even at pH = 7.4, by prolonged dialysis against very large quantities of physiological sodium chloride solution. Dialysis against distilled water, at pH = 7.4, left a residue of 25 to 45 per cent of the calcium bound to the protein; but this could be liberated if the pH were reduced to 2.5 (Loeb). "The difficulty or impossibility of freeing serum proteins from calcium by electrodialysis at the isoelectric points of the proteins is well known" (109), and merely indicates that the acid- and base-combining powers are neutralised to an equivalent extent at the isoelectric point of the ampholyte. Only when the pH is greatly to the acid side, or when more powerful bases (e.g. Na) are present, can the calcium be driven off in its entirety. The isoelectric points of serum albumin and serum globulin are 5.5 and 4.4 (pH values) respectively (137). The total base-combining powers of the serum proteins, as determined by van Slyke, Hastings, Hiller, and Sendroy (177) at pH = 7.35, were computed to be *a*, albumin—0.273 mM. per litre, and *b*, globulin—0.189 mM. per litre. With an average albumin:globulin ratio of 1:1.8, the B protein value for whole serum would be 0.243 mM. per litre. Greenberg, Larson, and Tufts (48) suggested a practical means of estimating the calcium-combining power of serum protein by subtracting from the total non-diffusible calcium content the determined value of the calcium in any non-diffusible phosphate ($Ca_3P_2O_8$) present. Their data (on beef serum) enable a formulation of the amount of combined calcium in terms of the known protein content and determined values for diffusible calcium (assumed to be identical with calcium ion concentration—v. infra).

The equation is

$$\frac{\text{Protein}}{\text{Ca-proteinate}} = 0.51 + \frac{3.5}{\text{Diffusible Ca}}$$

It is based on the mass action law and is asserted to be a slight improvement upon that of McLean and Hastings (109) who assumed maximal calcium (base) combining values.

Salvesen and Linder (152) observed a parallelism between (total) calcium and protein in sera from nephritis patients and in certain

dropsical fluids from cardiac cases. Numerous confirmatory data on nephritic and allied cases have led to several attempts to formulate the interdependence of calcium, protein, and inorganic phosphate on a mathematical basis, exemplified in the nomogram of Peters and Eiserson (136). It has also been pointed out (155), not without qualification (186, 167), however, that the calcium content of the interrelated body fluids (blood, lymph, cerebrospinal fluid, etc.) closely parallels their protein content. On the other hand, extensive analysis of sera from *normal* persons at various ages and from cases other than renal and cardiac (167, 186, 21) quite fail to establish any physiological dependence of total or diffusible calcium upon the serum protein content! Under normal in vivo conditions, therefore, it cannot be conceded that even the proportion of diffusible: total calcium is a simple function of the serum protein content, although, according to the data of McLean and Hastings (109), "it appears that the *ionization* of calcium in the fluids of the body is determined chiefly by the protein content of the fluids, and that the relationship between calcium and protein can, as a first approximation, be described by a simple mass law equation, yielding the ionization constant of calcium proteinate." Schmidt and Greenberg recalled that the calcium in nephrosis cases, and certain types of jaundice and neoplastic diseases, follows the albumin rather than the total protein. On fractionating the serum proteins by ammonium sulphate precipitation (31) about twice as much calcium was found in the (dialysed) albumin fraction than in the corresponding globulin. Graded membranes, which permitted the passage of all the albumin but very little globulin, demonstrated that practically the whole of the calcium accompanied the more-easily filtered (albumin) fraction (7).

Lipids. Watchorn and McCance (1932) in a detailed investigation of a large number of human sera, failed to find any correlation between diffusible (ultra-filtrable) calcium values and serum protein content. They suggested the possibility that organic lipoid phosphoric acids might be the colloids with which the non-diffusible calcium was bound. Loeb and Nichols (101) found that human sera shaken twice with equal volumes of ether averaged about 10 per cent more combined calcium in dialysis experiments, but discounted such variations. Wadsworth, Maltaner and Maltaner (182, 183) have demonstrated the ability of calcium to form stable compounds with such phospholipids as purified cephalin. Their preparations, however, only became active in hastening the clotting of recalcified oxalate plasma when the calcium had been removed by treatment with acid and subsequent dialysis. Rona and

Melli's (145) neglected observation that "lecithin" altered the permeability of collodion membranes in calcium partition experiments must also be recalled. In view of the evidence concerning the interrelationships of calcium and phospholipid (cephalin) in blood coagulation (v. infra), these inadequate data are in urgent need of further investigation.

Other calcium colloids. A colloidal form of tertiary calcium phosphate ($Ca_3P_2O_8$) has received considerable attention in the literature, and can indeed be demonstrated in blood under certain artificial conditions. However, Greenberg and Tufts (49) came to the conclusion that it would be too unstable to exist in blood with *normal* levels of calcium and inorganic phosphate. This colloidal compound must not be confused with the diffusible calcium-phosphorus complex discussed in a subsequent paragraph.

B. NATURE OF DIFFUSIBLE CALCIUM. Until very recently, the view has prevailed that only about one-third to one-half of the diffusible serum calcium actually exists in the simple ionised condition. Estimations of ionic calcium (discussed by Schmidt and Greenberg, 1935) have been beset with practical difficulties because of the interfering effects of *a*, other electrolytes, and *b*, the proteins present. Not one of the electrode, adsorption, or solubility data methods can stand up to the criticism of an alert physical chemist. Yet, for some unexplained reason, most of the methods have consistently yielded values for "ionised" calcium averaging about 2.0 mgm. per cent. The cited reviewers (155) see great significance in the trend of more recent data to present higher values, approximating those for the whole of the diffusible calcium fraction.

Thus Shear, Washburn and Kramer (160), and Shear and Kramer (159, 161) used $CaHPO_4$ crystals in a series of solubility determinations and obtained ionic calcium values of 6.6 mgm. per cent. The implicit assumption of a unit activity coefficient in the calculations based on their solubility data undoubtedly causes this figure to be somewhat in excess of the true value (155). The *biological method*, recently elaborated by McLean and Hastings (108), is an excellent means of estimating calcium-ion content, provided that (11) the effect on the frog heart be attributable solely to the calcium-ion, a conclusion which seems warranted by the absence of augmentation with calcium citrate and calcium proteinate (60). Results (4.4–6.4 mgm. per cent, 109), on a variety of biological fluids, indicate that such calcium as is diffusible and uncombined (with citrate, for instance) is present entirely as calcium-ions!

A typical experiment on calcium partition in horse serum read as follows: 1, total serum protein, 7.0 per cent; 2, albumin:globulin ratio = 1.8; 3, total serum calcium = 2.90 mM. per kgm. H_2O; 4, ionised calcium = 1.29 mM. per kgm. H_2O; 5, calcium combined with globulin = 0.465 mM. per kgm. H_2O; 6, calcium combined with albumin = 0.805 mM. per kgm. H_2O; 7, calcium unaccounted for = 0.34 mM. per kgm. H_2O. As the albumin values were felt to be slightly lowered by partial denaturation of the protein, there was evidently very little calcium left over for the possible diffusible but non-ionised forms about which so much has appeared in the recent literature. Schmidt and Greenberg (155) accepted the new data so wholeheartedly as to state: "a very large part of the evidence offered for the existence of calcium compounds other than the ordinary calcium-ion in the diffusible fraction of blood serum is either insufficient, incorrectly interpreted, or quite incorrect. This statement applies both to the organic citrate-like compound and the special non-ionised compounds of calcium phosphate."

The hypothetical *calcium-phosphorus complex* has been supported, in particular, by Benjamin and Hess (8–10) on the basis of adsorption experiments with barium sulphate. Notwithstanding the foregoing criticisms, Benjamin (11) demands further investigation of the experimental data before yielding her case.

Calcium citrate. Greenwald (51) suggested a citrate-like form of diffusible but non-ionised calcium. Since the introduction of Thunberg's (174) method for citric acid determinations in biological material, the evidence (Nordbö and Schersten, 126) has been against the presence of all but a variable trace (1–4 mgm. per cent) of citrates in normal sera. This could not account for more than about 1 mgm. per cent of calcium. Hastings, McLean, Eichelberger, Hall, and da Costa (60) have recently reported experiments with the frog heart technique, demonstrating clearly that calcium citrate is by no means non-ionised, but exists largely in the form of a negatively charged complex ion, representing an almost complete primary dissociation according to the equation

$$Ca_3Cit_2 = Ca(Ca\ Cit)_2 \rightleftharpoons Ca^{++} + 2\ Ca\ Cit^{-}$$

The secondary dissociation, being that of a weak electrolyte, is very feeble, and follows the equation

$$Ca\ Cit^{-} \rightleftharpoons Ca^{++} + Cit^{=}$$

Using the biological method to determine Ca^{++}, the dissociation constant, pK_{CaCit^-} (at 22°C., pH = 7.4, and total ionic strength of $\mu 0.155$–$\mu 0.163$) was computed at $3.22^{\pm}$ 0.025.

The scanty data in the literature on calcium citrate conductivity were quoted by the above authors in support of their views that such complex citrate-ions do exist. Explicable on this hypothesis also is the observation of Greenberg and Greenberg (45), confirmed by Peretti (133), that citrates (over 100 mgm. per cent) caused calcium to migrate to the anode in the electrodialysis of serum and serum ultrafiltrates. By preventing the accumulation of acid at the anode it was possible to avoid the appearance of calcium in the anodal compartment, thus contradicting the evidence of Bernhard and Beaver (12), Klinke (83) et al., who sought confirmation for the citrate-like compound in similar data on ultrafiltrates.

In further support of the calcium-citrate anion hypothesis, Ferguson and Du Bois (37) have recorded that citrated blood samples, kept in mercury tonometers and repeatedly tested for pH (glass-electrode method) over a period of hours, showed a greater acidity than defibrinated blood or samples received immediately into the electrode chamber and allowed to clot (no pH change being observed!) during the series of potentiometer readings. The higher acidity of the citrated specimens persisted during the glycolytic acid shift and also after aëration.

Conclusion. Emphasising the latest viewpoint, viz., that the diffusible calcium exists chiefly or solely in the ionised form, Schmidt and Greenberg (155) conclude: "even if a low calcium ion activity in serum is admitted, it is qualitatively to be ascribed to an explanation familiar with modern theories of strong electrolytes, namely, the result of the depressing action of the interionic influence of electrolytes and proteins of the blood."

Peters and van Slyke (137) refer to the theoretical and experimental support for the contention that "calcium in physiological concentration will precipitate from salt solutions containing concentrations of phosphate and bicarbonate (*of the order of that*) found in the blood." The blood plasma (or serum) must, therefore, be regarded as having a "stabilised supersaturation" of calcium under physiological conditions. "It appears to be established that the presence of an adequate amount of protein in serum is one of the conditions essential for the maintenance of the normal serum calcium concentration" (137).

Little clinical significance would seem to attach to variations, per se, in calcium content due to alterations in the amount of protein. A

possible exception, insufficiently studied because of the rarity of true cases, might be the "haemophilia calceopriva" described by Hess (65). Øwre's (129) comments on portal thrombosis should also be mentioned. Tetany, with hypocalcemia (whether in rickets, parathormone deficiency, high intestinal fistula, etc.) appears to be a function of the diffusible calcium and is not accompanied by blood coagulation anomalies (163).

The necessity of calcium for blood clotting. Hammarsten (1875–1877) noted that calcium chloride sometimes caused the formation of fibrin from otherwise non-clotting, or incompletely clotting, hydrocele fluids. Green (1887) had an inkling of the rôle of calcium in blood clotting when he found that calcium sulphate facilitated the coagulation of diluted "salt plasma." Ringer and Sainsbury (1890) noted that calcium, barium, and strontium chlorides counteracted the delay in clotting producible by mixing blood with sodium or potassium chloride solutions. Arthus and Pagès (1890) are, however, justly credited with the first clear demonstration that calcium salts are essential for in vitro coagulation. They added to blood (dog, ox, horse) 1, oxalates, 0.1 per cent; 2, fluorides, 0.15 per cent, and 3, soaps of the alkali metals, 0.5 per cent, and found that the blood remained fluid for several days. On the addition of calcium chloride solution, clotting was restored. Strontium could be substituted, but not barium (an error due, according to Mellanby (112), to the weak solutions employed).

Pekelharing (1891) gave an important early discussion of the rôle of calcium in relation to the other known factors in blood clotting (v. infra). In his monograph on "fibrin ferment" (thrombin) in the following year (1892) he recorded the first use of the powerful anticoagulant action of sodium citrate, 10 cc. of a 5 per cent solution being sufficient for 90 cc. of horse's blood. The preservation of fluidity he attributed to "the affinity of lime for the citric acid." Schmidt (1895) argued that there could not be a specific decalcification since calcium citrate, unlike the oxalate, etc., was not precipitated. This evoked a spirited defense by Arthus in the following year (1896). Sabbatini (1900–1903) settled the controversy by interpreting the rôle of calcium in terms of the ionic state (a view that has recently survived certain criticisms-v. infra). In the study of a dozen substances preventing blood coagulation by inactivating the calcium factor, he distinguished between *a*, those that precipitated calcium out of solution, and *b*, those that did not. He made extensive investigations with trisodium citrate and by means of careful analyses was able to determine that the in-

hibition of clotting of dog's blood in vitro required an excess of citrate, a minimum of three molecules of the re-crystallised citrate being needed for each atom of calcium present. The same proportion was found necessary to antagonise the ammonium oxalate precipitation of calcium chloride solutions. Four or five times as much citrate was required if given in vitro (intravenously) and all the dogs in these experiments suffered toxic symptoms which he ascribed to acute calcium deprivation. Confirming the untimely terminated experiments of Horne (1896), Sabbatini found that clotting was inhibited by an excess of calcium, the maximal concentration for clotting being about 18 grams of $CaCl_2$ per liter (molecular concentration = 0.162). Mellanby (1909) showed that calcium was a much more potent clot-aiding agent than the other alkaline earth metals, but strontium, and also barium, to some extent, could act in a similar manner. He, too, confirmed Horne's discovery of the anti-coagulant effects of excess of calcium salts, as did Rettger in the same year (1909). It may be noted that Stassano and Daumas (1924) observed a minimal coagulant concentration of calcium between 13 and 21 mgm. per liter of blood, and that Lewisohn (1915–1924), pioneer of the citrate method for human transfusions, redetermined the minimal anti-coagulant concentration of sodium citrate to be 0.15 to 0.20 per cent (i.e., considerably under the toxic intravenous dose which, for the average human adult, is about 5 grams). That doses of citrate, bordering on the toxic, may be given intravenously to *shorten* coagulation time and perhaps control internal hemorrhage (nevertheless, at a definite risk) is apparent from the numerous data in the clinical literature.

CALCIUM CONTENT OF THE PLASMA PROTEINS. Hammarsten (58, 59) credited Brücke (20) with the observation that washed fibrin always contained an appreciable trace of calcium (0.064 per cent, Frederickse, 42) in its ash. Making use of fibrinogen solutions from which the calcium had been removed as completely as possible by oxalation and centrifugation, Hammarsten obtained clots containing as little as 0.005 per cent of Ca (in terms of dry weight of fibrin). This would require an unreasonably high molecular weight (circa 800,000) if calcium were assumed to be part of the fibrin molecule. Although these "decalcified" reagents interacted rather slowly, the high potency of thrombin (Schmidt) is such that Hammarsten's experiments failed to rule out the possibility of calcium being an integral part of the coagulant (Howell, 73). Csapó and Faubl's (31) analytical data on horse blood plasma are summarised in table 1. It may be noted that the three protein fractions

were precipitated from 0.3 per cent citrate plasma by *a*, 28 per cent; *b*, 50 per cent, and *c*, full saturation with ammonium sulphate, and dialysed for four weeks against distilled water.

de Waele (184) studied the solubility of fibrinogen preparations in terms of associated salts, pH, and lipids. Two minima of solubility were noted, one near pH = 4.0, the other near pH = 7.0. Fibrinogen itself, in contradistinction to its salts, was isolelectric at pH = 6.9 and was incoagulable by heat. It could combine with alkali metals and various acids to form "fibrinogenates" of varying solubility and coagulability. The peculiar *gel* formation so characteristic of blood clots was said to occur only with the fibrinogenate of calcium, but coagulation did not occur after extraction of the lipids unless cephalin (or peptone or gelatin) were added subsequently "to facilitate the entrance of calcium ions into the fibrinogen complex."

There seems to be some doubt concerning the *isoelectric points* of fibrin and fibrinogen (Howell, ·73, 74). Vonk (181) determined the

TABLE 1

	FIBRINOGEN	SERUM GLOBULIN	SERUM ALBUMIN
Concentration in plasma (dry weights per 100 cc.)	0.4 gram	2.8 grams	4.0 grams
Calcium content (per cent)	31.01 mgm.	37.78 mgm.	77.8 mgm.

isoelectric point of fibrin (the washed dried clot from horse and ox blood) from the swelling minima in various buffered solutions. The readings were found to be affected not only by pH but also by other anions and cations. If HCl and NaOH, only, were used to vary pH, a value of 7.1 was obtained, but the end point could easily be displaced by various buffers to as acid a value as pH = 3.7. Klinke and Ballowitz (84) recorded the following isoelectric points: *a*, fibrinogen, pH = 4.20 – 4.54; *b*, fibrin (whether obtained by coagulation or heat precipitation), pH = 5.23 – 5.66. Mainzer (106) employed 1, cataphoresis (zone of minimal migration), and 2, suspension instability (solubility minimum or zone of maximal flocculation) in experiments on a 0.1 per cent solution (in 1 per cent NaCl) of human blood fibrinogen. The following data were obtained with *a*, buffered, and *b*, unbuffered solutions: 1, *a*, pH = 5.15, *b*, pH = 5.43; 2, *a*, pH = 4.36, *b*, pH = 4.90. The corresponding values after denaturation with alcohol were 1, *a*, 5.0, *b*, 5.55; 2, *a*, 4.0, *b*, 4.5. The behaviour of fibrinogen was by no means that of an ideal ampholyte.

Fischer (41) used turbidity estimations as a measure of the suspension instability (flocculation) in confirming the slight shift of the isoelectric point of fibrinogen during *denaturation*. Prothrombin (serum or muscle globulin) behaved quite differently. Numerous agencies (e.g., long standing, shaking, heat, actinic influences, acids and alkalies, and "thrombokinase"—including purified brain phospholipid) all had the power to denature prothrombin, the process being accompanied by a marked shift in the isoelectric point (as determined by the zone of maximal flocculation or turbidity increase) from the original value of pH = 5.3 to near neutrality. This non-specific denaturation could be prevented (but not reversed) by heparin. *A further change, believed to be the formation of a globulin + lipid + calcium complex (v. infra) was necessary for the elaboration of a "thrombin" (coagulant) capable of clotting fibrinogen.*

Howell (72) found that both fibrinogen and thrombin (a highly purified preparation freed from phosphorus (calcium analyses not given) and dissolved in weak NaCl solution) had isoelectric points between pH = 4 and 5, as determined by zone of maximal flocculation on heating and on radiation. The purified thrombin showed some cathodal migration in cataphoresis experiments, but in serum and oxalated plasma the thrombin and prothrombin exhibited a negative charge, as did the plasma fibrinogen. In certain experiments with cell-free oxalated plasma and sodium chloride (0.9 per cent) in the anode and cathode compartments, fibrinogen was detected in both tubes, especially at pH close to neutrality. The electro-positive fraction gave a flocculent or membranous clot with thrombin; the electro-negative portion yielded a gelatinous clot with a variable extent of the typical ultramicroscopic needle formation (the "crystalline gel" obtainable with ordinary fibrinogen and the non-migrating fraction). "These results suggest that the fibrinogen particles or aggregates may adsorb both hydrogen and hydroxyl ions." Howell (74) showed that Kugelmass' (87) earlier attempts to elucidate the thrombin-fibrinogen interaction on the basis of electrical adsorption were insufficiently supported by valid experimental facts. He also (75) criticised del Baere's (6) cataphoresis data from which an electropositive thrombin had been deduced. It is obviously desirable that new electrochemical data be obtained on better purified (38) fibrinogens.

Eagle and Baumberger (33) and Ferguson and DuBois (37) could find no evidence of any pH change during clotting.

Pending a more complete elucidation of the physico-chemical aspects

of coagulation, the exact part played by calcium remains uncertain. We can, however, review the progress which has been made in the classical enquiry concerning the rôle of calcium in the activation of the coagulant factor (s), which is now generally recognized to be the earlier of the two major phases (Hammarsten) of the clotting reactions.

CALCIUM AND THE ACTIVATION OF COAGULANTS. A. *Prothrombin.* Pekelharing (1892) demonstrated that calcium salts were essential for the activation of prothrombin, the globulin fraction of the plasma proteins that serves as the precursor of the coagulant which Schmidt had termed thrombin (fibrin ferment). This activation of prothrombin by calcium salts was confirmed by Morawitz (121) and all subsequent workers, notably including Howell (op. cit.), Bordet and Delange (15, 16), Wöhlisch and Paschkis (187). The most direct evidence in the classical data is the easily confirmed observation that prepared prothrombin does not coagulate "decalcified" plasmas, exudates, or purified fibrinogen solutions, but will do so after the addition of calcium salts. Recalcified prothrombin (which may be termed Ca-prothrombin) can clot fibrinogen-containing fluids even in the presence of excess of oxalate, etc., thus suggesting that ionic calcium is no longer necessary after the coagulant has been activated.

Stewart and Percival (169) found that a 24-hour *partial* precipitation of serum calcium by oxalate involved both the diffusible and non-diffusible fractions. Oxalates, citrates, fluorides, and excess of sodium chloride added to plasma were said to cause an early disappearance of "ionised" calcium, whereas lengthening of clotting time appeared later, pari passu with an increase in diffusible calcium, complete inhibition of clotting coinciding with maximal values for diffusible calcium (approximating the total calcium content). Schulten's (157) criticisms of the Brinkman-van Dam (18) method invalidate the conclusion that *complete* depression of the calcium ion could be achieved by subminimal amounts of "decalcifying" anticoagulants. In view of the cited data (158, 178) on the activation of prothrombin by small amounts of added calcium in the presence of oxalate, it is more in keeping with the classical evidence to suggest that ionic calcium was not ruled out in Stewart and Percival's experiments. In such case, calcium ions would be "available" *a*, readily, at first, until the whole of the diffusible calcium was immobilized; and *b*, less readily, subsequently, owing to slower dissociation of the plasma calcium-proteinates.

Quantitative relationships between calcium and decalcifying anticoagulants. We have already noted that ordinary blood clotting requires

the presence of a (total) calcium content of not less than 13 to 21 mgm. per litre (166), nor more than about 18 grams per litre (0.162 M $CaCl_2$, 149).

Calcium as an anticoagulant. A number of authorities (68, 112, 150, 141), have been cited as demonstrating the anticoagulant effects of *large amounts* of calcium salts. While this has not been elucidated, it may be recalled that excess of any neutral salt preserves the fluidity of the blood, and that modifications of the stability of protein solutions accompany the presence of sufficient quantities of electrolytes. If this explanation is correct Ca should fall into place in an Hofmeister series.

Von Zárday (1933) restudied the vitro optima, minima, and maxima of both calcium and citrate in blood coagulation. He noted that the anticoagulant effects of excess of calcium could be overcome by the subsequent addition of citrates.

Decalcifying anticoagulants. Sabbatini (148–150) found that the amount of trisodium citrate necessary to prevent blood clotting constituted a decided excess. In vitro, the minimal requirement of anticoagulant, according to careful analyses, corresponded closely to 3 molecules of citrate for each atom of calcium present. In vivo, several times more citrate was required, probably owing to its oxidative destruction in the body (Salant and Wise, 151). Peretti (133–135) noted a dependence upon pH.

Clowes and West (26–28) found that blood coagulation was prevented by a proportion (chemical equivalents) of citrate: calcium in excess of 70:30 (cf. Bloch, 14). Very similar proportions of citrate were required also to neutralise the actions of calcium salts in *a*, lessening the number of drops of watery soap solution running from a stalagmometer into a column of olive oil; *b*, killing mice when injected subcutaneously, and *c*, causing interference with the specific hemolysis of red blood cells by complement and amboceptor (anti-complementary Wassermann reaction). A common explanation for these divergent phenomena was sought for in the dependence of all of them upon colloidal equilibria of lipids in the presence of inorganic salts, the simplest example being the well known phase reversal of sodium soap emulsions on the addition of calcium salts. This hypothesis, unfortunately, is still far in advance of our factual knowledge of the colloidal phenomena which occur during clotting, but it may assist in the explanation of platelet lysis (v. infra).

Vines (178, 179) pointed out that only 0.61 to 0.66 per cent, or one-seventeenth, of the available (total serum) calcium was needed to

restore the normal coagulation time of finger-prick blood which had been prevented from clotting by the use of minimal amounts of oxalate, citrate, or fluoride. This was true only if the blood were freshly oxalated. "Some process of firmer union with the oxalate" (? v. infra), which was completed in about an hour, raised the minimal calcium requirement to 2.03 per cent. At the completion of this period, subsequent additions of oxalate (over and above the *minimum*) could be neutralised by approximately equivalent amounts of calcium. These facts form the basis of an ingenious "clotting test" method (the term "biological" is to be deprecated) for the determination of serum calcium values (within an error of about 3 per cent). Vines' own conclusions especially with regard to ionic calcium have since been proved erroneous, but his experimental facts have been verified (188, 158). He may be relied upon also for the observations that *a*, saturated solution of calcium citrate could clot oxalated blood, although it could not (in reasonable amounts) prevent the coagulation of normal blood; and *b*, carbonates did not prevent clotting albeit "the calcium ion in $CaCO_3$ is more depressed than in CaF_2" —Vines dismissed, overhastily, the current suggestion that ionisable bicarbonates would be formed. Mellanby (113) noted that prothrombin ("prothrombase") could be activated in the presence of $CaCO_3$. His preparations were apt to show some "spontaneous" thrombin formation (114).

Scott and Chamberlain (158) added varying amounts of calcium to minimally oxalated dog plasma and confirmed Vines' data by finding that some clotting recurred when a mere $\frac{1}{20}$ of the oxalate present was re-neutralised by added calcium chloride. Quantitative estimations of the amount of fibrin formed on progressively restoring the "calcium deficit" indicated an increased fibrin formation pari passu with the calcium restoration. The parallelism was by no means a simple proportion but it did suggest that "when inorganic calcium is added to decalcified plasma the decalcified thrombin can compete at first against potassium oxalate for the possession of calcium and active thrombin is formed. The active thrombin exerts its action for some time even in the presence of oxalate until it is again inactivated by the loss of calcium."

Whether or not the decalcifying anticoagulants can themselves combine with the plasma colloids (Stuber and Sano, 172; Scheuring, 153) is still an unsettled problem, but it is suggested, inter alia, by *a*, the excess of minimal anticoagulant amounts over the chemical equivalents of calcium, and by *b*, Vines' observation (v. supra) that blood which has

been standing in contact with oxalate requires a larger "minimum" of calcium to restore coagulation.

Calcium in prothrombin and thrombin preparations. Not only is ordinary *thrombin* a product of altered plasma (the calcium content of which has already been discussed) but the conditions of its formation ordinarily include the presence of ionised calcium at least to the stage of prothrombin activation (*vide* Ca-prothrombin) and often to the completion of the clotting process, when the thrombin is prepared from the fibrin or the fresh serum. Without raising the question of antithrombin, we may concede that the fluidity of the circulating blood is sufficient evidence that active thrombin is not normally present in the intravascular blood. Moreover, thrombin cannot be prepared from fresh blood received directly into alcohol (Schmidt, 154) although the alcohol takes down with the proteins some 61 per cent (90) to 82 per cent (24) of the total serum calcium. Fibrin washed until calcium-free (Loucks and Scott, 103) gave negative results with the usual techniques for thrombin extraction. Restoration of calcium *prior* (but not subsequent) to precipitation resulted in potent extracts in the experiments on blood and plasma (26, 103). Eagle's (1935) quantitative studies have demonstrated clearly that *a*, thrombin formation preceded clotting; *b*, the amount of thrombin formed is dependent primarily on the amount of prothrombin activated (Eagle did not vary the concentration of the activating agents sufficiently to give weight to his statement "the amount of thrombin ultimately formed. . . . is independent of the amount of Ca, platelets, or cephalin used"), and *c*, the activation of prothrombin by calcium salts shows a peculiar latency succeeded by a rapid phase of completion. Cephalin and platelets diminish the latency and speed up the activation.

While the considerable body of evidence, which we have reviewed, supports the conclusion that thrombin is normally the product of the action of ionised calcium upon a precursor substance present in the blood plasma (a view that was clearly formulated before the turn of the century) it is yet difficult to give a final answer to any of the following pertinent questions: 1. Is the mode of action of calcium a definite and stable colloidal combination or merely a catalysis of a process (? denaturation) which can take place in its absence? 2. Can prothrombin ever be activated by *combined* calcium when conditions of depression of the calcium ion prevail? 3. If thrombin *is* a calcium compound, does it owe its coagulant properties to that fact, and by what mechanism (catalysis, chemical affinity, favouring adsorption, by shifting isoelectric

points, or in other ways)? A brief presentation of relevant arguments may be considered.

1. Notwithstanding the impossibility of freeing blood proteins from combined calcium without resort to drastic treatment (v. supra), no effort has been made (as far as we are aware) to study quantitatively the combined calcium content of the numerous thrombin and prothrombin preparations which have been used in coagulation experiments. Howell (1910–1913) prepared a thrombin (from fibrin) which, at the expense of considerable loss in potency, could be obtained in a form which resisted heat coagulation for short periods owing, 'twas said, to paucity of inorganic constituents. Forty years ago, Hammarsten (op. cit.) tried to remove as much calcium as possible by oxalation of both fibrinogen and coagulants. The obtaining of a fibrin with a very low calcium content was (as he admitted) no criterion of the significance of otherwise small calcium residua for the activity of the *thrombin*. Kastl's (80) recent preparation of an active thrombin by the alcohol precipitation of fresh (beef) serum after filtering off the calcium oxalate formed in 24 to 48 hours by the Kramer-Tisdall-Schimmelpfeng technique, has been supplemented by calcium analyses (189) from which it was claimed that calcium-free thrombin could clot Ca-free fibrinogen. No demonstrations of the so-called "spontaneous" transformation of prothrombin into thrombin or the activation of purified prothrombin by other means than recalcification (see p. 449 of Howell's 1935 review) record calcium analyses on either the coagulant or (more especially) the test solutions of fibrinogen. Rabinovich's (140) electrodialysis experiments (v. supra) stand alone as evidence for the inactivation of the clotting mechanism by an allegedly thorough decalcification (reversible by the addition of inorganic calcium). We except the insufficiently substantiated oxalation data of Loucks and Scott (103).

2. While it is universally asserted that plasma kept in contact with decalcifying anticoagulants retains its fluidity, it is a common experience, nevertheless, to find traces of fibrin deposited on long keeping (even in the ice-chest under sterile conditions). Cited experiments on progressive (169) and regressive (178, 158) oxalation suggest that some coagulation can occur under conditions of considerable depression of calcium ionisation. The coagulant-activating powers of Ca-citrate (178) and $CaCO_3$ (113) point in the same direction.

3. Data supporting the view that thrombins are calcium-cephalin-protein compounds will be reviewed in the succeeding section.

B. *Tissue coagulants.* L. Loeb (95–97) and Mills (115–120) showed

that calcium salts were necessary for the clotting of fibrinogen by tissue coagulants (coagulins), even under conditions thought to preclude the ordinary thrombin mechanism. Smith, Warner, and Brinkhouse (164) used fibrinogen, freed from prothrombin by Fuchs' (43) method of $Mg(OH)_2$ adsorption, to substantiate a claim that watery extracts of fresh beef lung, that had been thoroughly perfused, were devoid of coagulant activity on recalcification. Quick (139) asserted confirmatory evidence. Loucks and Scott (103) had previously noticed a great diminution of activity if the lungs were perfused prior to preparing an alcohol-precipitated extract. This was correlated with a diminished calcium content, the weakest extract containing only 0.3 mgm. per cent of Ca. The best coagulants were prepared from lung extracts to which $CaCl_2$ was added just before the alcohol precipitation. Dialysis did not lessen the activity of potent extracts, but oxalation did so. Ferguson (38) made an experimental analysis of the coagulant properties of *a*, plasma prothrombin; *b*, platelets; *c*, corneal-, and *d*, crystalline-lens extracts (the last two chosen because of physiological absence of admixed blood). It was concluded that all four coagulant precursors required for their activation *a*, calcium and, equally essential, *b*, cephalin (which might already be present in "available" form). In all cases the optimal thrombic activity was obtained under conditions suggesting certain quantitative interrelationships between three component factors, namely, 1, protein (prothrombin-like); 2, phospholipid (cephalin-like), and 3, calcium.

Calcium in relation to the phospholipid factor. The literature on blood coagulation (188) contains abundant evidence of the importance of phospholipid, thought by Zak (191), Howell (70), McLean (110), and most subsequent workers (81), to be *cephalin*, or, at least (194, 41) closely associated with that phosphatid. Mills (115), adduced reasons for differentiation between "free" (dissociable) cephalin (the thermostable lipid) and "combined" cephalin (the thermolabile lipoproteins of tissues, termed "thrombokinase" or "thromboplastin" by earlier workers). Both forms are demonstrable in platelets and in most tissue extracts. The exact rôle of phospholipid in the various phases of the clotting process is still sub judice, chiefly owing to the prevalence of Howell's view that the *sole* function of these "thromboplastic agents" is to neutralize the clot inhibitory actions of heparin (antiprothrombin) and antithrombin. The data of Mills (119, 120) based on the earlier experiments of Bordet (15, 16), have been confirmed and extended by Ferguson (38), and it would appear difficult to escape the conclusion

alluded to above, namely, that cephalin, as well as calcium, is a sine qua non in the *direct* activation of all coagulants. "Until it has been shown possible to prepare a phospholipid-free prothrombin able to clot *phospholipid-free* fibrinogen on simple recalcification, we must conclude that thrombin is a definite calcium-cephalin-prothrombin (complex or) compound." Wadsworth, Maltaner, and Maltaner (182, 183), Fischer (41), and Wu (190) arrived at the same conclusion. Less direct evidence for an interrelationship between calcium, phosphatid, and prothrombin appears in a number of other cited references (103, 178, 184, 26).

Conclusion. The weight of the foregoing evidence supports the current conclusion that ionised calcium is essential for the formation of the coagulant factor (thrombin) from its inactive precursors in the blood and tissues. Whether or not we can subscribe to the attractive possibility that thrombin is a colloidal calcium-cephalin protein compound is still an open question, and the exact mechanism of the fibrinogen-thrombin interaction has not yet been elucidated.

The "decalcifying" anticoagulants would appear to act not only by the simple depression of calcium ionisation but also in a more complex manner involving a splitting-off of calcium from its colloidal combinations, with the additional possibility (172, 153) that the oxalate, etc., may itself enter into union with the plasma colloids.

Calcium and anticoagulants. The inability of calcium salts to clot heparinised, hirudinised, "peptone", and other similar anticoagulant bloods demonstrates that these preservers of plasma fluidity act in some other manner than on the calcium mechanism.

A possible rôle of calcium (64, 178, 41) in the neutralisation of these clot-inhibiting agents by phospholipids (including thrombokinase) is insufficiently substantiated.

Calcium in relation to platelet lysis. Haurowitz and Sládek (61), working with *horse* blood, gave the following calcium determinations (mgm. per cent): *a*, platelets—0.074; *b*, leucocytes—0.04; *c*, erythrocyte stroma—0.064. The respective lipid contents (cholesterol in parentheses) were *a*, 12 (1.7); *b*, 22 (7.4), and *c*, 10.2 (1.5 mgm. per cent.

The "preservation" of platelets in decalcified (oxalated, etc.) blood was noted by a number of workers (cited, 34). Ferguson (1934) reinvestigated the dark-field appearance of platelet "alterations" and reviewed the evidence in support of the conclusion that "the surface-tension lowering action of a wettable surface is the primary cause of platelet alteration. This leads to the formation of *excrescences* differing

in type according to whether clotting ensues or is prevented by depression of the calcium ion function." A final concentration of about 0.25 per cent of trisodium citrate is necessary to inhibit platelet lysis (rabbit blood) and this "critical concentration" is practically uninfluenced by the factor of osmotic tonicity. "A further change supervenes in order to liberate from the platelets the essential factor for fibrin formation. This change has been shown to be a process of osmotic imbibition and partial plasmolysis in which the essential (specific) rôle is played by the calcium ions. It is reasonable to suppose that the expanded platelet excrescence is especially liable to osmotic influences." The similarity of platelet alterations to the *myelin figures* (Virchow, 1854; Leathes, 1925) seen when phosphatides come into contact with watery electrolytes, is worthy of record, especially in connection with the dark-field observation (34) of penetration of lipoid (lecithin) films by calcium salt solutions in the physical type of phase distribution alluded to by Clowes (op. cit.). This suggests the reason why water *enters* the platelets to cause the swelling and lysis described. It is an established fact (138) that platelet lysis alone (by saponin, for instance) is not necessarily accompanied by coagulation if other factors in the clotting mechanism are in abeyance, e.g., *a*, inactivation of the calcium by oxalates, citrates, etc. (v. supra), and *b*, under conditions imposed by powerful antithrombic factors, which, according to both Howell and Mills, have marked ability to deviate phospholipid whether in "free" or "combined" form.

The answer to the pertinent question as to why the circulating blood remains fluid in the known presence of calcium ions and the fibrin factors (which are all part of the plasma itself) is to be sought for in a consideration of the physico-chemical conditions necessary for coagulation. Howell's theory centers round the ability of heparin and antithrombin (demonstrably present in normal plasma) *a*, to prevent the calcium activation of prothrombin, and *b*, to neutralise any formed thrombin. Ferguson (38) believes that the evidence for the essential rôle of cephalin also in the *direct* activation of coagulants necessitates a somewhat revised viewpoint. The function of the antithrombic factors may be interpreted as that of protectors of plasma against various situations (physiological and pathological) in which cephalin (the phospholipid factor) may become "available" for acting, in conjunction with calcium, to form thrombin (coagulant) from the ever-present plasma prothrombin, with the attendant risk of intravascular clotting. This "cephalin availability theory" of blood clotting directs attention to the sources of cephalin for initiating coagulation. The rôle of lysed platelets and

injured tissues in this connection has received adequate consideration. A further possibility, latent in the data of Fischer (41) on "denaturation" of the plasma globulins and of Ferguson (38) on the additional cephalin rendered available by boiling (heat denaturation) of coagulant lipoproteins, is that an important liberation of phospholipid may accompany the irreversible changes undergone by shed plasma. Thus, we envision a definite physico-chemical advance toward the conclusion (rather obscurely presented by Pickering, 1928) that blood coagulation is a phenomenon which owes its inception to a disruption of the normal stabilised equilibrium of the entire colloidal "plasma complex."

REFERENCES

(1) Abderhalden, E. Physiological Chem. (Trans. W. T. Hall and G. Defren) 1911 edn. pp. 554–555 (tables cited).

(2) Alport, A. C. Biochem. J. **18**: 455, 1924. The accurate estimation of calcium in whole blood.

(3) Anderson, G. H. Brit. J. Dis. Child. **21**: 33, 107, 1924. The calcium and phosphorus content of the blood in normal and rachitic children.

(4) Arthus, M. and C. Pagès. Arch. de physiol. norm. et path. (Ser. 5) **2**: 739, 1890. Nouvelle theorie chimique de la coagulation du sang.

(5) Arthus, M. Arch. de physiol. norm. et path. (Ser. 5) **47**: 47, 1896. La coagulation du sang et les sels de chaux. (Réfutation expérimentale des objections d'Alexander Schmidt).

(6) del Baere, L. J. Biochem. Ztschr. **246**: 38, 1932. Über den Mechanismus der Blutgerinnung.

(7) Bendien, W. M. and I. Snapper. Biochem. Ztschr. **260**: 105, 1933. Untersuchungen über die Bindung der Kolloide des Serums mit Hilfe von Ultrafiltern erhöhter Durchlässigkeit; besteht im Serum eine komplexe Albumin-Globulinverbindung? Ist das gebundene Calcium des Serums an Albumin oder an Globulin gebunden?

(8) Benjamin, H. R. and A. F. Hess. J. Biol. Chem. **100**: 27, 1933. The forms of calcium and inorganic phosphorus in human and animal sera. I. Normal, rachitic, hypercalcaemic, and other conditions.

(9) Benjamin, H. R. J. Biol. Chem. **100**: 57, 1933. II. The nature and significance of the filtrable, adsorbable calcium-phosphorus complex.

(10) Benjamin, H. R. and A. F. Hess. J. Biol. Chem. **103**: 629, 1933. III. A comparison of physiological and experimental hypercalcaemia.

(11) Benjamin, H. R. J. Biol. Chem. **109**: 123, 1935. IV. A reply to Greenberg and Larson.

(12) Bernhard, A. and J. J. Beaver. J. Biol. Chem. **69**: 113, 1926. The electrodialysis of human blood serum.

(13) Birch, C. la F. Proc. Soc. Exper. Biol. and Med. **28**: 752, 1931. Haemophilia.

(14) Bloch, M. The Lancet, 301, 1920. Coagulation of the blood: a study of the anticoagulant action of sodium citrate and the part played by calcium in the blood.

(15) BORDET, J. AND L. DELANGE. Ann. Inst. Pasteur **27**: 341, 1913. Sur la nature du cytozyme, recherches sur la coagulation du sang.

(16) BORDET, J. AND L. DE LANGE. Ann. et bull. Soc. roy. d. sc. méd. et nat. de Bruxelles **72**: 87, 1914. Analyse et synthèse du processus de la coagulation.

(17) BORDET, J. Bull. Johns Hopkins Hosp. **32**: 213, 1921. (Herter lecture.) The theories of blood coagulation.

(18) BRINKMAN, R. AND E. VAN DAM. Proc. Kon. Akad. Wetensk. Amsterdam **22**: 762, 1920. (Abstr. in Physiol. Abstr. **5**: 272, 1920. Estimation of ion concentration in ultrafiltrates and other protein-free solutions.)

(19) BROWN, A., I. F. MACLACHLAN AND R. SIMPSON. Am. J. Dis. Child. **19**: 413, 1920. The effect of intravenous injections of calcium in tetany and the influence of cod liver oil and phosphorus in the retention of calcium in the blood.

(20) BRÜCKE, E. Virchow's Arch. **12**: 81, 172, 1857. Ueber die Ursache der Gerinnung des Blutes.

(21) BÜLL, H. Biochem. Ztschr. **216**: 228, 1929. Über den Einfluss der Fällungsdauer auf die direkte Bestimmung des Calciums im Blutserum und über die Grösse des dialysablen und des adialysablen Anteiles.

(22) CAMERON, A. T. AND V. H. K. MOORHOUSE. J. Biol. Chem. **63**: 687, 1925. The tetany of parathyroid deficiency and the calcium of the blood and cerebrospinal fluid.

(23) CANTAROW, A. Calcium metabolism and calcium therapy. 2nd ed., 1933.

(24) CERNATESCO, R. AND I. ORNSTEIN. Compt. rend. Soc. de biol. **115**: 786, 1934. Sur le calcium et le sodium protéiques.

(25) CLARK, G. W. J. Biol. Chem. **49**: 487, 1921. The microdetermination of calcium in whole blood, plasma, and serum by direct precipitation.

(26) CLOWES, G. H. A. AND F. WEST. Proc. Soc. Exper. Biol. and Med. **11**: 6 (et al.), 1913. On the rôle played by antagonistic ions in the process of blood coagulation.

(27) CLOWES, G. H. A. J. Physic. Chem. **20**: 407, 1916. Protoplasmic equilibrium.

(28) CLOWES, G. H. A. Am. J. Physiol. **42**: 610 (proc.), 1916. On the mechanism of blood coagulation.

(29) COWIE, D. M. AND H. A. CALHOUN. J. Biol. Chem. **37**: 505, 1919. The presence of calcium in the red blood corpuscles of ox and man.

(30) CRUICKSHANK, E. W. H. Biochem. J. **17**: 13, 1923. Studies in experimental tetany. (I) On the distribution of calcium in the plasma and cells. (II) On the variations in colloidal and ionic calcium.

(31) CSAPÓ, J. AND J. FAUBL. Biochem. Ztschr. **150**: 509, 1924. Calciumgehalt der Serumeiweissfraktionen.

(32) EAGLE, H. J. Gen. Physiol. **18**: 531, 547, 813, 1935. Studies on blood coagulation. I. The rôle of prothrombin and of platelets in the formation of thrombin. II. The formation of fibrin from thrombin and fibrinogen. IV. The nature of the clotting deficiency in haemophilia.

(33) EAGLE, H. AND J. P. BAUMBERGER. J. Gen. Physiol. **18**: 809, 1935. Studies on blood coagulation. III. On the constancy of the hydrogen ion concentration during the coagulation of fibrinogen by thrombin.

(34) FERGUSON, J. H. Am. J. Physiol. **108**: 670, 1934. Observations on the alterations of blood platelets as a factor in coagulation of the blood.

(35) FERGUSON, J. H. Proc. Soc. Exper. Biol. and Med. **31**: 929, 1934. The cephalin content of prepared fibrinogen and prothrombin solutions.

(36) FERGUSON, J. H. Blood: I. Blood and lymph; II. Blood coagulation. (Review, in B. HARROW AND C. P. SHERWIN'S Textbook of biochemistry.) 1935.

(37) FERGUSON, J. H. AND D. DU BOIS. J. Lab. Clin. Med. **21**: 663, 1936. Observations on the pH of clotting and citrated blood.

(38) FERGUSON, J. H. Am. J. Physiol. (in press), 1936. An experimental analysis of coagulant activation.

(39) FINDLAY, L., D. N. PATON AND J. S. SHARPE. Quart. J. Med. **14**: 352, 1921. Studies in the metabolism of rickets. I. Is rickets due to a deficient supply of calcium to the bones? II. The calcium balance in normal and rachitic children.

(40) FISCHER, A. Jap. J. Exper. Med. **13**: 223, 1935. Studies on coagulation of blood.

(41) FISCHER, A. AND CO-WORKERS. Numerous papers in Biochem. Ztschr. esp. (I) **259**: 67. Über den Reaktionsmechanismus der Blutgerinnung. (II) **264**: 169, 178, 184. Über die chemische Natur des Thrombins. I. Fraktionierung und Anreicherung. II. Die Komponenten des Thrombins. III. Die Küpplung der Thrombinkomponenten. (III) **280**: 235, 250. Über die Wirkung von Säure auf die Eiweissvorstufe des Thrombins. Über die Aktivierung des Prothrombins. 1933, etc.

(42) FREDERICKSE, J. J. Compt. rend. Soc. de Biol., 10-S., 1894. I. 415. De l'existence de calcium dans la fibrine.

(43) FUCHS, H. J. Ztschr. f. Immunitätsforsch. u. exper. Therap. **59**: 424, 1928. Ueber die Beteiligung des Komplements bei der Blutgerinnung. IV. Der Serozymgehalt der Blutplättchen, eine neue Gerinnungstheorie.

(44) GREEN, J. R. J. Physiol. **8**: 354, 1887. On certain points connected with the coagulation of blood.

(45) GREENBERG, D. M. AND L. D. GREENBERG. J. Biol. Chem. **99**: 1, 1932. Is there an unknown compound of the nature of calcium citrate present in the blood?

(46) GREENBERG, D. M. AND L. GUNTHER. J. Biol. Chem. **85**: 491, 1929–1930. On the determination of diffusible and non-diffusible serum calcium.

(47) GREENBERG, D. M. AND C. E. LARSON. J. Biol. Chem. **109**: 105, 1935. Evidence of adsorption experiments on the forms of calcium and inorganic phosphorus in blood serum.

(48) GREENBERG, D. M., C. E. LARSON AND E. V. TUFTS. Proc. Soc. Exper. Biol. and Med. **32**: 647, 1935. Colloidal calcium phosphate of blood and calcium partition in serum.

(49) GREENBERG, D. M. AND E. V. TUFTS. J. Biol. Chem. **105**: xxxii (proc.), 1934. Formation of colloidal form of calcium phosphate in blood stream in experimental hypercalcaemia.

(50) GREENE, C. H. AND M. H. POWER. J. Biol. Chem. **91**: 183, 1931. The distribution of electrolytes between serum and the *in vivo* dialysate.

(51) GREENWALD, I. J. Biol. Chem. **67**: 1, 1926. The effect of the administration of calcium salts and of sodium phosphate upon the calcium and phophorus metabolism of thyroparathyroidectomised dogs, etc.

(52) GUILLAUMIN, C. O. Bull. soc. chim. biol., **12**, 1269, 1930. Constatations expérimentales sur la mesure du calcium sanguin après separation oxalique. Sa repartition entre les globules, et la plasma ou le sérum.

(53) VON GULÁCSY, Z. Biochem. Ztschr. **251**: 162, 1932. Über den Calciumgehalt der roten Blutkörperchen im menschlichen Blute.

(54) HALVERSON, J. O. AND O BERGEIM. J. Biol. Chem. **32**: 159, 1917. The determination of small amounts of calcium, particularly in blood.

(55) HALVERSON, J. O., H. K. MOHLER AND O. BERGEIM. J. Biol. Chem. **32**: 171, 1917. The calcium content of the blood serum in certain pathological conditions.

(56) HAMMARSTEN, O. Nova Acta Reg. Soc. Sci. Upsaliensis, Ser. 3, **10**: 1, 1875. (Abstr. in R. MALY'S Jahresbericht ü. d. Fortschr. f. Tier-Chem., 1875, **5**, 19. Untersuchungen über die Fäserstoffgerinnung.)

(57) HAMMARSTEN, O. Pflüger's Arch. **14**: 211, 1877. Zur Lehre von der Fäserstoffgerinnung.

(58) HAMMARSTEN, O. Hoppe-Seyler's Ztschr. f. physiol. Chem., **22**: 333, 1896. Ueber die Bedeutung der löslichen Kalksälze für die Fäserstoffgerinnung.

(59) HAMMARSTEN, O. Hoppe-Seyler's Ztschr. f. physiol. Chem. **28**: 98, 1899. Weitere Beiträge zur Kenntnis der Fibrinbildung.

(60) HASTINGS, A. B., F. C. MCLEAN, L. EICHELBERGER, J. L. HALL AND E. DA COSTA. J. Biol. Chem. **107**: 351, 1934. The ionization of calcium, magnesium, and strontium citrates.

(61) HAUROWITZ, F. AND J. SLÁDEK. Hoppe-Seyler's Ztschr. f. physiol. Chem. **173**: 233, 1928. Über die chemische Zusammensetzung der Blutplättchen.

(62) HEIDUSCHKA, A. AND H. SCHMIDT-HEBBEL. Biochem. Ztschr. **253**: 336, 1932. Vergleichende Bestimmungen des Kalkgehaltes in den verschiedenen Blutbestandteilen unter verschiedenen Bedingungen ihrer Gewinnung.

(63) HERBERT, F. K. Biochem. J. **27**: 1975, 1978, 1933. Calcium of whole blood, serum and plasma in human diseases, including tetany. Total and diffusible calcium of serum and calcium of cerebrospinal fluid in human cases of hypocalcaemia and hypercalcaemia.

(64) HESS, A. F. Proc. Soc. Exper. Biol. and Med. **13**: 59, 1915–1916. An interrelationship between calcium and antithrombin in blood coagulation.

(65) HESS, A. F. Arch. Int. Med. **17**: 203, 1916. The blood and blood vessels in hemophilia and other hemorrhagic diseases.

(66) HITCHCOCK, D. I. AND R. B. DOUGAN. J. Gen. Physiol. **18**: 485, 1935. Freezing points of anticoagulant salt solutions.

(67) HOLT, G. W. Proc. Soc. Exper. Biol. and Med. **29**: 315, 1932. Heparin in blood calcium analyses.

(68) HORNE, R. M. J. Physiol. **19**: 356, 1896. The action of calcium, strontium and barium salts in preventing coagulation of the blood.

(69) Howell, W. H. Am. J. Physiol. **26**: 453, 1910. The preparation and properties of thrombin, together with observations on antithrombin and prothrombin.

(70) Howell, W. H. Am. J. Physiol. **31**: 1, 1912. The nature and action of the thromboplastic (zymoplastic) substance of the tissues.

(71) Howell, W. H. Am. J. Physiol. **35**: 474, 1914. Prothrombin.

(72) Howell, W. H. Am. J. Physiol. **40**: 526, 1916. Structure of the fibrin gel and theories of gel-formation.

(73) Howell, W. H. The Harvey Lectures. Ser. XII, 272, 1916–1917. The coagulation of the blood.

(74) Howell, W. H. Proc. Inst. Med. Chicago **5**: 139, 1924–1925. (Pasteur lecture.) The problem of coagulation.

(75) Howell, W. H. Physiol. Rev. **15**: 435, 1935. Theories of blood coagulation.

(76) Jansen, W. H. Hoppe-Seyler's Ztschr. f. physiol. Chem. **101**: 176, 1917. Kalkstudien am Menschen. I. Zur Methodik der Bestimmung des Blutkalks.

(77) Jansen, W. H. Deutsch. Arch. f. klin. Med. **125**: 168, 1918. Kalkstudien am Menschen. II. Der Kalkgehalt des Menschlichen Blutes.

(78) Jones, M. R. and L. L. Nye. J. Biol. Chem. **47**: 321, 1921. The distribution of calcium and phosphoric acid in the blood of normal children.

(79) Jones, M. R. J. Biol. Chem. **49**: 187, 1921. The calcium content of plasma and corpuscles in the new born.

(80) Kastl, O. Biochem. Ztschr. **274**: 452, 1934. Ist das Thrombin eine Calciumverbindung.

(81) Kuwashima, Y. J. Biochem. (Japan) **3**: 91, 1924. Studies on some factors in the coagulation of blood.

(82) Kirk, E., W. H. Lewis, Jr. and W. R. Thompson. J. Biol. Chem. **111**: 641, 1935. The effect of age on the plasma calcium content of men.

(83) Klinke, K. Biochem. Ztschr. **213**: 177, 1920. Neue Beiträge zur Frage des Calciumzustandes in den Körperflüssigkeiten.

(84) Klinke, K. and K. Ballowitz. Ztschr. f. d. ges. exper. Med. **84**: 224, 1932. Gerinnumgsstudien; der isoelektrische Punkt des Fibrins.

(85) Kramer, B. and F. F. Tisdall. J. Biol. Chem. **47**: 475, 1921. A simple technique for determination of calcium and magnesium in small amounts of serum.

(86) Kramer, B. and F. F. Tisdall. J. Biol. Chem. **53**: 241, 1922. Distribution of sodium, potassium, calcium, and magnesium between corpuscles and serum of human blood.

(87) Kugelmass, I. N. Arch. internat. de Physiol. **21**: 139, 1923. Contribution physicochemique au mécanism de la coagulation du sang.

(88) Leathes, J. B. The Lancet, 957, 1925. (Croonian lecture.) The rôle of fats in vital phenomena.

(89) Leiboff, S. L. J. Biol. Chem. **85**: 759, 1930. The absence of calcium in the human red blood cells.

(90) Levy, J. and F. Gallais. Bull. Soc. Chim. Biol. **15**: 1330, 1933. Protéides et cations du sérum sanguin.

(91) LEVY, J. AND M. M. PACU. Bull. Soc. chim. biol. **15**: 1317, 1933. Ultrafiltration du sérum et du plasma. Teneur des ultrafiltrats de serum et de plasma en chlore, potassium, sodium, calcium, magnesium.

(92) LEWISOHN, R. Med. Record (N. Y.) **87**: 141, 1915. A new and greatly simplified method of blood transfusion.

(93) LEWISOHN, R. Annal. Surg. **64**: 618, 1916. The importance of the proper dosage of sodium citrate in blood transfusion.

(94) LEWISOHN, R. Boston Med. and Surg. J. **190**: 733, 1924. The citrate method of blood transfusion after ten years—A retrospect.

(95) LOEB, L. Biol. Bull. **8**: 240, 1904–1905. Coagulation of the blood.

(96) LOEB, L. Am. J. Physiol. **18**: *xvii* (proc.), 1907. The action of blood serum and tissue extracts on the coagulation of the blood.

(97) LOEB, L., M. S. FLEISCHER AND L. TUTTLE. J. Biol. Chem. **51**: 461, 485, 1922. Interaction between blood serum and tissue extract in coagulation of the blood, etc.

(98) LOEB, R. F. J. Gen. Physiol. **8**: 451, 1925. The effect of pure protein solutions and of blood serum on the diffusibility of calcium.

(99) LOEB, R. F. AND E. G. NICHOLS. Proc. Soc. Exper. Biol. and Med. **22**: 275, 1925. The influence of proteins on the diffusibility of calcium.

(100) LOEB, R. F. AND E. G. NICHOLS. J. Biol. Chem. **72**: 687, 1929. Factors influencing the diffusibility of calcium in human blood serum.

(101) LOEB, R. F. AND E. G. NICHOLS. J. Biol. Chem. **74**: 645, 1927. Effects of dialysis and of ether extraction on the diffusibility of calcium in human blood serum.

(102) LOUCKS, M. M. AND F. H. SCOTT. Am. J. Physiol. **76**: 186 (proc.), 1926. The rôle of calcium in the coagulation of blood.

(103) LOUCKS, M. M. AND F. H. SCOTT. Am. J. Physiol. **91**: 27, 1930. Calcium in the coagulation of blood.

(104) LYMAN, H. J. Biol. Chem. **29**: 169, 1917. A rapid method for determining calcium in blood and milk.

(105) LYMAN, H. J. Biol. Chem. **30**: 1, 1917. The calcium content of human blood.

(106) MAINZER, F. Klin. Wchnschr. **11**: 1382, 1932. Der isoelektrische Punkt des genuinen und denaturierten menschlichen Fibrinogens in gepufferter und ungepufferter Losung.

(107) MAINZER, F. Biochem. Ztschr. **246**: 164, 182, 1932. Der isoelektrische Punkt des genuinen Fibrinogens. Der isoelektrische Punkt des denaturierten Fibrinogens.

(108) MCLEAN, F. C. AND A. B. HASTINGS. J. Biol. Chem. **107**: 337, 1934. A biological method for the estimation of calcium ion concentration.

(109) MCLEAN, F. C. AND A. B. HASTINGS. J. Biol. Chem. **108**: 285, 1934. The state of calcium in the fluids of the body. I. The conditions affecting the ionization of calcium.

(110) MCLEAN, J. Am. J. Physiol. **41**: 250; **43**: 586, 1916–1917. The thromboplastic action of cephalin. The relation between the thromboplastic action of cephalin and its degree of unsaturation.

(111) MARRACK, J. AND G. THACKER. Biochem. J. **20**: 580, 1926. The state of calcium in the body fluids.

(112) MELLANBY, J. J. Physiol. **38**: 28, 441, 1908–1909. The coagulation of the blood.

(113) MELLANBY, J. Proc. Roy. Soc. (Ser. B) **107**: 271, 1930. Prothrombase—Its preparation and properties.

(114) MELLANBY, J. Proc. Roy. Soc. (Ser. B) **113**: 93, 1933. Thrombase—Its preparation and properties.

(115) MILLS, C. A. J. Biol. Chem. **46**: 135, 167, 1921. The chemical nature of tissue coagulins. The action of tissue extracts in the coagulation of blood.

(116) MILLS, C. A. AND A. P. MATHEWS. Arch. internat. de Physiol. **24**: 73, 1924. Les deux mécanismes physiologique de la coagulation du sang.

(117) MILLS, C. A. Am. J. Med. Sci. **172**: 501, 1926. Considerations of the problem of blood clotting.

(118) MILLS, C. A. Am. J. Physiol. **76**: 632, 1926. Blood clotting studies in haemophilia.

(119) MILLS, C. A. Chinese J. Physiol. I; several papers incl. (i) 123: Period of induction in blood clotting or sequence of events preceding fibrin formation (ii) 235: Rôle of platelets in blood clotting. (iii) Clotting properties of pure blood and of pure plasma. (iv) Is cephalin necessary in the activation of prothrombin? 1927.

(120) MILLS, C. A. Am. J. Physiol. **95**: 1, 1930. Do blood platelets, plasma, and tissues yield thrombin, or tissue fibrinogen?

(121) MORAWITZ, P. Deutsch. Arch. f. klin. Med. **79**: 1, 215, 432, 1903–1904. Beiträge zur Kenntnis der Blutgerinnung.

(122) MORAWITZ, P. Ergebn. d. Physiol. **4**: 307, 1905. Die Chemie der Blutgerinnung.

(123) MORAWITZ, P. 1925. Blutgerinnung. (In C. OPPENHEIMER's Handbuch der Biochemie des Menschen und der Tiere. 2 ed., **4**: 44, 1925).

(124) MORGULIS, S. AND A. M. PERLEY. J. Biol. Chem. **88**: 169, 1930. Studies on cerebrospinal fluid and serum calcium with special reference to the parathyroid hormone.

(125) MULLI, K., T. PILLEWIZER AND W. LAVES. Arch. exper. Path. u. Pharmakol. **172**: 384, 1933. Über die Reaktionen einiger blutgerinnungshemmender Stoffe mit Calcium-Ion in wässeriger Lösung.

(126) NORDBÖ, R. AND B. SCHERSTÉN. Skand. Arch. f. Physiol. **63**: 124, 1931. Bestimmung der zitronensäure im Blut mit der Thunbergmethode und mit der Pentabromazeton methode.

(127) NORTHROP, J. H. AND M. KUNITZ. J. Gen. Physiol. **7**: 25, 1924. The combination of salts and proteins. I.

(128) NORTHROP, J. H. AND M. KUNITZ. J. Gen. Physiol. **9**: 351, 1925. The combination of salts and proteins. II. A method for the determination of the concentration of combined ions from membrane potentials.

(129) ØWRE, A. Acta Chir. Skand. **65** (Suppl. 13); 1, 1929. Studies of the clinic and etiology of post-operative thrombosis.

(130) ØWRE, A. Med. Rev. **48**: 49, 1931. (Abstr. in Chem. Abstr. **26**: 4089, 1932. The difference between the calcium contents of plasma and serum.)

(131) PEKELHARING, G. A. Internat. Beiträge z. wiss. Med. (Festschr. R. Virchow), **1**: 433, 1891. Ueber die Bedeutung der Kalksälze für die Gerinnung des Blutes.

(132) PEKELHARING, G. A. Untersuchungen über das Fibrinferment. (Amsterdam), 1892.

(133) PERETTI, G. Arch. Sci. Biol. Napole **17**: 126, 1932. Contributio allo studio della reazione fra radicale citrico e Ca-ions. (Abstr. in Physiol. Abstr. **18**: 89, 1933–1934.)

(134) PERETTI, G. Boll. soc. ital. biol. sper. **8**: 1645, 1933. (Abstr. in Chem. Abstr. **28**: 2736, 1934. The electrophoresis of calcium in blood serum and its dialysate.)

(135) PERETTI, G. Boll. soc. ital. biol. sper. **9**: 780, 782, 785, et seq., 1934. (Abstr. in Chem. Abstr., **29**: 187, et seq., 1935.)

(136) PETERS, J. P. AND L. EISERSON. J. Biol. Chem. **84**: 155, 1929. The influence of protein and inorganic phosphorus on serum calcium.

(137) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. I. Interpretations. Vol. II. Methods, 1935.

(138) PICKERING, J. W. The blood plasma in health and disease, 1928.

(139) QUICK, A. J. Am. J. Physiol. **114**: 282, 1936. On various properties of thromboplastin (aqueous tissue extracts).

(140) RABINOVICH, R. Compt. Rend. Soc. de biol. **93**: 1635; **95**: 1180, 1926. Contribution a l'étude de la coagulation du sang.

(141) RETTGER, L. J. Am. J. Physiol. **24**: 406, 1909. The coagulation of blood.

(142) RINGER, S. AND H. SAINSBURY. J. Physiol. **11**: 369, 1890. The influence of certain salts upon the act of clotting.

(143) RONA, P. AND D. TAKAHASHI. Biochem. Ztschr. **31**: 336, 1911. Über das Verhalten des Calciums in Serum und über den Gehalt der Blutkörperchen an Calcium.

(144) RONA, P. AND D. TAKAHASHI. Biochem. Ztschr. **49**: 370, 1913. Beitrag zur Frage nach dem Verhalten des Calciums im Serum.

(145) RONA, P. AND G. MELLI. Biochem. Ztschr. **166**: 242, 1925. Beitrag zur Frage der Ionenberteilung im Blutserum III.

(146) ROTHWELL, C. S. J. Biol. Chem. **74**: 257, 1927. The determination of calcium in whole oxalated blood.

(147) RYMER, M. R. AND R. C. LEWIS. J. Biol. Chem. **95**: 441, 1932. Studies on the calcium content of human blood corpuscles.

(148) SABBATINI, L. Atti della Reale Accad. delle Sci. di Torino **36**: 18, 1900–1901. (Abstr. in Mosso's Arch. ital. de biol. **36**: 397, 1910. Calcium et citrate trisodique dans la coagulation du sang, de la lymph, et du lait.)

(149) SABBATINI, L. Memorie della Reale Accad. delle Sci. di Torino, (Ser. 2) **51**: 267, 1901. (Abstr. in Mosso's Arch. ital. de biol. **36**: 416, 1901. Fonction biologique du calcium. I. Action antagoniste entre le citrate trisodique et le calcium.)

(150) SABBATINI, L. Memorie della Reale Accad. delle Sci. di Torino (Ser. 2) **52**: 213, 1902–1903. (Abstr. in Mosso's Arch. ital. de biol. **39**: 333, 1903. II. Le Calcium dans la coagulation du sang.)

(151) SALANT, W. AND L. E. WISE. J. Biol. Chem. **28**: 27, 1916. The action of sodium citrate and its decomposition in the body.

(152) SALVENSEN, H. A. AND G. C. LINDER. J. Biol. Chem. **58**: 617, 635, 1923. Observations on the inorganic bases and phosphates in relation to the protein of blood and other body fluids in Bright's disease and in heart failure. The relation between calcium and protein of serum in tetany due to parathyroidectomy.

(153) SCHEURING, H. Biochem. Ztschr. **277**: 437; **279**: 436; **283**: 1, 1935. Über den Calcium-Stoffwechsel in der ersten Phase der Blutgerinnung.

(154) SCHMIDT, A. Zur Blutlehre. (Leipzig), 1892.
Weitere Beiträge zur Blutlehre. (Wiesbaden), 1895.

(155) SCHMIDT, C. L. A. AND D. M. GREENBERG. Physiol. Rev. **15**: 297, 1935. Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism.

(156) SCHÖNBERGER, S. Biochem. Ztschr. **251**: 167, 1932. Über den Calciumgehalt der roten Blutkörperchen im menschlichen Blute.

(157) SCHULTEN, H. Biochem. Ztschr. **164**: 47, 1925. Zur Bestimmung der freien Ca-ionen nach Brinkman und van Dam.

(158) SCOTT, F. H. AND C. CHAMBERLAIN. Proc. Soc. Exper. Biol. and Med. **31**: 1054, 1934. Calcium and coagulation of blood.

(159) SHEAR, M. J. AND B. KRAMER. J. Biol. Chem. **79**: 105, 121, 125, 1928. Composition of bone. I. Analytical micro methods. II. Pathological calcification. III. Physicochemical mechanism.

(160) SHEAR, M. J., M. WASHBURN AND B. KRAMER. J. Biol. Chem. **83**: 697, 1929. VII. Equilibration of serum solutions with dicalcium phosphate.

(161) SHEAR, M. J. AND B. KRAMER. J. Biol. Chem. **86**: 677, 1930. IX. Equilibration of serum with dicalcium phosphate.

(162) SHELLING, D. H. AND H. L. MASLOW. J. Biol. Chem. **78**: 661, 1928. The effect of sodium citrate, acetate, and lactate on the ultrafiltrability of serum calcium.

(163) SIMPSON, S. AND A. T. RASMUSSEN. Quart. J. Exper. Physiol. **10**: 145, 1916. Does thyroparathyroidectomy in the dog affect the blood coagulation time?

(164) SMITH, H. P., E. D. WARNER AND K. M. BRINKHOUSE. Am. J. Physiol. **107**: 63, 1934. Lung extract and blood clotting.

(165) STANFORD, R. V. AND A. H. M. WHEATLEY. Biochem. J. **19**: 710, 1925. The estimation of calcium in the blood.

(166) STASSANO, H. AND A. DAUMAS. Compt. rend. Acad. Sci. **150**: 937, 1924. Du rôle double du calcium dans la coagulation du sang et de la lymphe.

(167) STEARNS, G. AND G. C. KNOWLTON. J. Biol. Chem. **92**: 639, 1931. Lack of relationship between calcium protein, and inorganic phosphorus of serum of non-nephritic children.

(168) STEWART, C. P. AND G. PERCIVAL. Biochem. J. **21**: 301, 1927. Studies in calcium metabolism. I. The action of parathyroid hormone on the calcium content of the serum and on the absorption and excretion of calcium.

(169) STEWART, C. P. AND G. PERCIVAL. Biochem. J. **22**: 548, 559, 1928. II. The calcium content of corpuscles, plasma and serum. III. Calcium and the coagulation of blood.

(170) STEWART, C. P. AND G. PERCIVAL. Physiol. Rev. **8**: 283, 1928. Calcium metabolism.

(171) STUBER, B. Die Blutgerinnung. (In H. Hirschfeld and A. Dittmair's Handbuch der allgemeinen Hämatologie **2**: 1145, 1934.)

(172) STUBER, B. AND M. SANO. Biochem. Ztschr. **134**: 260, 1922–1923. Untersuchungen zur Lehre von der Blutgerinnung. VII. Ueber die Rolle des Kalks bei der Blutgerinnung.

(173) THOMSON, D. L. AND J. B. COLLIP. Physiol. Rev. **12**: 309, 1932. The parathyroid glands (esp. pp. 328–335 on "the physical state of the blood calcium").

(174) THUNBERG, T. Am. J. Physiol. **90**: 540, 1929. Citric acid in animal fluids.

(175) UNDERHILL, F. P. AND A. DIMICK. J. Biol. Chem. **58**: 133, 1923. The metabolism of inorganic salts. IV. The content of inorganic salts in the blood in pregnancy, with especial reference to calcium.

(176) UNDERHILL, F. P. AND E. G. GROSS. J. Biol. Chem. **81**: 163, 1929. Is there a relationship between the spleen and calcium metabolism?

(177) VAN SLYKE, D. D., A. B. HASTINGS, A. HILLER AND J. SENDROY, JR. J. Biol. Chem. **79**: 769, 1928. Studies of gas and electrolyte equilibria in blood. XIV. The amounts of alkali bound by serum albumin and globulin.

(178) VINES, J. W. C. J. Physiol. **55**: 86, 287, 1921. The coagulation of the blood. I. The rôle of calcium. II. The clotting complex.

(179) VINES, J. W. C. The parathyroid glands in relation to disease. 1924.

(180) VIRCHOW, R. Virchow's Arch. **6**: 562. Ueber das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den thierischen Geweben.

(181) VONK, J. J. Hoppe-Seyler's Ztschr. f. physiol. Chem. **198**: 201, 1931. Quellungsminimum und isoelektrischer Punkt des Fibrins.

(182) WADSWORTH, A., F. MALTANER AND E. MALTANER. Am. J. Physiol. **80**: 502, 1927. A study of the coagulation of the blood; the chemical reactions underlying the process.

(183) WADSWORTH, A., F. MALTANER AND E. MALTANER. Am. J. Physiol. **91**: 423, 1930. Further studies of the chemical reactions underlying the coagulation of the blood. The activity of lecithin.

(184) DE WAELE, H. (cited by J. W. PICKERING). Abstr. 12th Physiol. Congress, 1926, 165. Ann. de physiol. et physicochem. biol. **3**: 94, 1927.

(185) WAELSCH, H., S. KITTEL AND A. BUSZTIN. 1935. Hoppe-Seyler's Ztschr. f. physiol. Chem. **234**: 27, 1935. Die Bewegung der Mineralstoffe zwischen Blutkörperchen und Plasma und die Grundformen der Permeation.

(186) WATCHORN, E. AND R. A. MCCANCE. Biochem. J. **26**: 54, 1932. Inorganic constituents or cerebrospinal fluid. II. The ultrafiltration of calcium and magnesium from human sera.

(187) WÖHLISCH, E. AND K. PASCHKIS. Klin. Wchnschr. **2**: 1930, 2319, 1923. Ein direkter Nachweis der spezifischen Rolle des Kalks bei der Entstehung des Thrombins. Addendum.

(188) WÖHLISCH, E. Ergebn. d. Physiol. **28**: 443, 1929. Die Physiologie und Pathologie der Blutgerinnung.

(189) WÖHLISCH, E. Personal communication. 1936.

(190) Wu, H. Proc. Soc. Exper. Biol. and Med. **32**: 97, 1934. Clotting of plasma in the absence of lipoid.

(191) Zak, E. Arch. f. exper. Path. und Pharmacol. **70**: 27, 1912. Studien zur Blutgerinnungslehre. I.

(192) von Zárday, I. Wien. Arch. inn. Med. **24**: 283, 1933. Kalziumoptimum und Blutgerinnung.

(193) von Zárday, I. Fol. haematol. **52**: 33, 1934. Blutgerinnungstudien. (Kalkproblem und allgemeine Salzwirkung).

(194) Zunz, E. and J. La Barre. Arch. internat. Physiol. **18**: 116, 1921. Recherches sur l'action des phosphatides dans la coagulation du sang.

EFFECTS PRODUCED BY THE IRRADIATION OF PROTEINS AND AMINO ACIDS

L. EARLE ARNOW

Division of Physiological Chemistry, University of Minnesota, Minneapolis

A. THE DENATURATION PROCESS. 1. *The Nature of the Process.* All workers in the field of protein irradiation have agreed that the most obvious effect of exposing protein solutions to radiant energy is the denaturation of the protein. This denaturation occurs in two distinct steps. Thus, Bovie (6, 7), Young (85), Galinsky (30), and Rajewsky (57, 58) all speak of 1, *a preliminary process,* in which the protein molecule becomes a new chemical entity, and 2, *a physical process,* causing the formation of a visible coagulum. Rajewsky further divides the second process into two portions: *a,* a conglomeration of the denatured particles to large visible masses, and *b,* a precipitation of the coarsely dispersed particles. This latter author found that the latent period before coagulation occurred in his experiments could be computed by means of the equation:

$$LZ = K\left(1 + \frac{1}{t}\right),$$

where LZ is the latent period (Latenzeit), K is a constant, and t is the time of exposure to the radiation. The equation applied only at constant temperature and constant radiation intensity. Gentner and Schwerin (31) reported that the intensity of radiation and the time of exposure required to give a constant latent period were related as expressed by the equation:

$$\frac{I}{I'} = \left(\frac{T'}{T}\right)^x$$

Where I and I' are radiation intensities, and T and T' are exposure times. In their experiments, the exponential, x, had an average value of 0.904.

Janet Clark (10, 11, 12, 13) has advanced the idea that the initial action of radiant energy on proteins is the production of photoelectric molecules; that is, that the radiation causes the emission of electrons

from the molecules of protein undergoing irradiation. This same author (14) recently has accumulated data which indicate that the denaturation of egg albumin by ultra-violet radiation occurs in *three* steps: 1, an alteration of the protein molecule by the radiation; 2, a reaction between the radiation-altered molecule and water, and 3, the formation of a visible coagulum.

a. *The action of heat as compared with that of radiation.* It was the contention of the early workers that the coagulum produced by the heat coagulation of a protein was identical with the precipitate resulting from the irradiation of the same protein. Rayband (59) observed that both protoplasm itself and protein isolated from it coagulated under the influence of ultra-violet light. He believed the process to be identical with that which occurred when the proteins were heat coagulated. Fernau and Pauli (25, 26) observed that many native proteins were coagulated irreversibly by the rays of radium, and they state that the process closely resembled the effect produced by heat. Schanz (60, 61) found that the changes produced in egg and serum albumins by exposure to heat, visible light, and ultra-violet light were readily comparable. Clark (13) believed that the coagulum produced by ultra-violet light and that produced by heat were identical, although she thought the mechanisms involved probably were different in the two cases. Fernau and Pauli (25) measured the changes in viscosity of a gelatin solution exposed to the penetrating rays of radium and found the results to be similar to those obtained when gelatin solutions were heated.

In more recent years, however, several papers coming from the University of Vienna have made it almost certain that the effect produced by heat is not the same as that produced by radiant energy. Spiegel-Adolf (62, 66) reported that electrolyte-free serum albumin coagulated under the influence of ultra-violet rays as under the influence of heat, but the coagulum could not, as in the latter case, be converted into a water soluble product by treatment with alkali and subsequent dialysis. Potassium thiocyanate did not aid in the resolution of the coagulum as it did in the case of heat coagulation. The dispersion coefficients (defined in a later section) for heated pseudoglobulin solutions were 1.88 to 2.00, whereas the same coefficients for solutions irradiated with ultra-violet light were 2.2 to 2.9. The characteristic wave length (also defined later) was the same for untreated and for heated pseudoglobulin solutions, but was increased for irradiated solutions. Fernau and Spiegel-Adolf (28) noticed that the coagulum resulting when serum albumin was coagulated with the rays of radium was not

alkali-soluble. Spiegel-Adolf and Krumpel (71) reported that the increased absorption capacities in the ultra-violet region of the spectrum resulting when albumins were heated, in the presence of acid or alkali to prevent coagulation, were qualitatively similar to those appearing after ultra-violet radiation, but were quantitatively very different. Spiegel-Adolf (67) found that ultra-violet radiation increased the refraction of light by solutions of the blood proteins; heat denaturation had no such effect.

Lieben and Ehrlich (43) found that halogens were split from artificially halogenated casein, diiodotyrosine, and thyroxin following exposure to ultra-violet rays. These results could not be reproduced by heating the same solutions for some time at 80°C.

b. *The action of alcohol and acetone as compared with that of radiation.* Kruyt and Bungenberg de Jong (38, 39) have offered good evidence to show that the denaturation of lyophilic sols by alcohol or acetone is due primarily to a removal of the water of hydration. This process appears to be purely a physical one; hence, if it is assumed that the effects produced by the irradiation of proteins are the same as the effects produced by adding acetone or alcohol to the same solutions, then it is necessary to assume that this process also is a physical dehydration.

Fernau and Pauli (25) suggested that the effects produced by irradiating native proteins with the rays of radium might be the same as the effects produced by adding alcohol to the solutions. Mond (50) noticed that the amount of alcohol necessary to precipitate albumins was less after irradiation with ultra-violet light than before. He does not maintain, however, that the addition of the alcohol was, in effect, simply a continuation of the same process initiated by the radiation. Similarly, both Schanz (61) and Young (85) have noticed that adding acetone or alcohol to an albumin solution before irradiation with visible and ultra-violet light hastens the denaturation process. It is not necessary, however, to interpret this finding as indicating a similarity in the action of the two agencies on the protein solutions. The action of the acetone or alcohol is probably best explained as a physical dehydration process in the case of visible radiation, and as a combination of dehydration with photodynamic action in the case of ultra-violet radiation.

c. *The action of oxidizing agents as compared with that of radiation.* Effront (19) seems to have been the first worker in the field of protein irradiation to suggest that the effects of irradiation might be due to a preliminary formation of hydrogen peroxide from water, the hydrogen peroxide thus formed actually producing the observed phenomena.

Fernau (23), investigating the effects of Röntgen rays, ultra-violet rays, and alpha-particles on albumin solutions, concluded that the effects produced were identical with those produced by means of hydrogen peroxide. Fernau and Spiegel-Adolf (28) noticed that the coagulum separating from a solution of serum albumin as a result of treatment with radium radiations could not be made water soluble by treatment with alkali. This same type of coagulum was produced if the albumin solution were treated with hydrogen peroxide. Galinsky (30) denatured gelatin with light and with chromates; she states that the reactions were strikingly similar. Meisling (49) noted that the denaturation of gelatin by light proceeded more rapidly if chromates were present. Lieben (42) states that the color reaction of Viosenet and Hopkins disappears after a period of irradiation of protein with ultra-violet light, but appears again if all traces of hydrogen peroxide are removed with spongy platinum.

2. *The Effect of Electrolytes.* It is well known that the addition of high concentrations of electrolytes to protein solutions will cause the proteins to become less stable. It might be expected, therefore, that the addition of electrolytes to protein solutions undergoing irradiation would accelerate the denaturation process. Bovie (6, 7) found that the coagulation of egg albumin solutions by means of ultra-violet rays was much more rapid in the presence of ammonium sulphate. Young (85) noticed that serum and egg albumin solutions coagulated more rapidly under the influence of sunlight or strong arc illumination when sodium chloride, ammonium sulphate, or potassium thiocyanate was present.

On the other hand, the addition of small quantities of salt does not appear to accelerate the process. Spiegel-Adolf (62, 63) reported that neutral salts did not prevent the coagulation of serum albumin by ultra-violet rays, but that the precipitation was prevented or inhibited by the presence of small amounts of polyvalent ions. The presence of salts arrested the denaturation of proteins by the rays of radium. Fernau and Spiegel-Adolf (27) observed that irradiation of pseudoglobulin solutions with radium led to a clumping together of the protein micelles. The radium apparently had no effect on salt solutions of pseudoglobulin.

It has been observed that the addition of electrolyte after a preliminary period of irradiation often will cause denaturation, whereas the addition of the same concentration of electrolyte before exposure to radiation had no apparent effect. Thus, Schanz (60, 61) found that albumins irradiated in acid solution with ultra-violet rays were more sensitive to ammonium sulphate and sodium chloride than they were

previous to the irradiation. Mond (50) later made the same observation. With the idea of testing her hypothesis that the initial action of radiation lies in the formation of photoelectric molecules, Clark (11, 12, 13) irradiated egg albumin solutions at different hydrogen ion concentrations. Theoretically, in solutions having a pH greater than that of the iso-electric point (pH 4.8), radiation would cause a loss in negative charge of the negatively charged albumin molecules. Some protein molecules would eventually acquire a positive charge, and mutual precipitation would occur. At pH 4.8 or less, the protein should be stabilized, since it naturally has a positive charge below pH 4.8. Normally, albumins below the iso-electric point are precipitated by half-saturation with ammonium sulphate, the ease of precipitation increasing as the pH decreases. On the other hand, very high concentrations are required to do this above the iso-electric point. After irradiation with ultra-violet rays, all of Clark's egg albumin solutions precipitated after half-saturation with ammonium sulphate, except solutions at pH 4.7, pH 4.8, and and pH 4.9, these solutions coagulating spontaneously under the influence of the radiation. Solutions below the iso-electric point precipitated more easily after addition of ammonium sulphate than did the other solutions.

In one important particular, the work of Schanz (60, 61) does not agree with that of Clark, just summarized. The former worker found that serum and egg albumin, in solutions with pH above that of the iso-electric point, deposited less precipitate upon addition of ammonium sulphate or sodium chloride after irradiation with ultra-violet rays than the same solutions did before such exposure.

3. *The Effect of Acidity and Alkalinity.* Clark (11, 12, 13), Young (85), Zhukov and Unkovskaya (86), Spiegel-Adolf (62, 64, 65), Arnow (1), and others have observed that the actual coagulation process will occur only if the pH of the protein solution is close to that of the iso-electric point. The addition of small quantities of acid or alkali will therefore protect against flocculation.

4. *The Importance of Sterility.* When a protein solution is invaded by a bacterial flora, the reaction of the solution, due to the production of ammonia and basic amines, becomes alkaline. It often happens that this alkalinity is sufficient to prevent the coagulation of the protein by radiation.

Spiegel-Adolf (63) noted that the velocity of coagulation of proteins exposed to ultra-violet radiation was markedly affected by the age of the protein solution after electrodialysis. Fernau and Spiegel-Adolf

(28) found that serum albumin, freshly dialyzed and preserved with toluence, lost its ability of being precipitated by radium irradiation unless it was preserved in an ice-box. Ammonia could be demonstrated in this serum albumin solution in amounts large enough to prevent the coagulation.

The observations recorded in the preceding paragraph led Spiegel-Adolf and Pollaczek (72) to make a more exhaustive study of the importance of sterility. Fourteen electrolyte-free solutions of proteins were prepared, and the relationship between sterility and coagulation velocity was studied. Germ-free solutions quickly coagulated, while infected solutions showed a marked delay, which persisted even after the organisms were dead. If sterile, quickly coagulating protein solutions were inoculated with bacteria, there was a marked delay before coagulation occurred. Electrodialysis of the protein solution which had been infected brought back the original coagulation velocity, although the bacteria were not removed by this process. The organism obtained from the protein solutions was almost always *Bacillus subtilis*, which apparently crowded out other organisms.

5. *The Effect of Photosensitizers.* Clark (12) has found that egg albumin solutions will coagulate under the influence of visible light in the presence of a suitable photosensitizer, such as eosin, erythrosin, rose bengal, methylene blue, or uranin. Lieben (41) succeeded in decomposing tyrosine and tryptophan with visible light in the presence of hematoporphyrin and rose bengal. Palit and Dhar (54) oxidized alanine and glycine by bubbling air through their aqueous solutions in the presence of sunlight, zinc oxide being utilized as a sensitizer. Meisling (49) found that the addition of chromates, aniline dyes, or chlorophyl to his gelatin solutions hastened the denaturation of the protein by visible and ultra-violet radiation. Neuberg (52) states that proteins and peptones were split by sunlight to amino acids, which then underwent reduction to aldehydes, the reaction occurring in the presence of uranium and iron salts.

As might be anticipated, however, the use of photosensitizers is not always successful in increasing the effects produced by radiant energy. Clark (12) reported that fuchsin and methyl orange, when added to egg albumin solutions, did not hasten the denaturation process. Ellinger (19) irradiated histidine with ultra-violet light and produced a substance which stimulated the intestine and caused a fall in blood pressure. He states that the addition of eosin and ferric chloride to his solutions did not hasten the reaction. Lieben and Jesserer (42) have noticed that the

denaturation of a Witte peptone solution by ultra-violet light is not affected by the addition of a photosensitizer.

6. *Miscellaneous Denaturation Experiments.* Apparently the first paper on the subject of protein irradiation was published by Hardy (32) in 1903. This author reported that acid solutions of ox serum globulin were rendered less opalescent by exposure of the solutions to radium bromide, whereas alkaline solutions of the same protein were coagulated. Hardy evidently was utilizing the alpha-particles, since thin mica filters prevented the effect.

Dreyer and Hanssen (16, 17) have reported that most of the common proteins are coagulated by ultra-violet light and by radium radiation. Stedman and Mendel (73) irradiated twelve purified proteins with ultra-violet light, and found that all the solutions developed the same characteristic odor, all were discolored yellowish, and all underwent a decrease in coagulation temperature. The appearance of a coagulum within solutions of proteins during irradiation was not universal and was characteristic for each protein.

Tian (80) observed that the surface of a gelatin gel was liquefied when the gel was exposed to ultra-violet rays.

Fernau and Spiegel-Adolf (28) found that the time required for precipitation of serum albumin with radium rays was almost independent of the concentration of the protein, except in solutions of very high concentration.

Arthus and Boshell (2) noted that Hammersten fibrinogen solutions deposited a precipitate during irradiation with ultra-violet light. It is doubtful, however, whether this represents a true denaturation process, since fibrinogen prepared by the authors did not deposit such a precipitate.

Mascherpa (48) reported that heavy doses of radium emanation caused a turbidity to appear in his egg albumin solutions, a result also noticed by Arnow (1).

B. Physical changes. 1. *Changes in Optical Rotation and in Dispersion Coefficients.* Since some of the terms which will be used in this section are not in common usage, the author thinks it best to define them here. The *dispersion coefficient*, or *dispersion quotient*, is defined as the ratio between the specific optical rotation at Fraunhöfer line F (4861 Å. U.) and the specific optical rotation at Fraunhöfer line C (6563 Å. U.). The curve resulting when the inverse of the specific optical rotation is plotted as a function of the square of the wave length (measured in microns) of light used in making the determinations, the temperature

being maintained constant, is the *specific dispersion isotherm.* The *characteristic wave length* is the square root of the abscissa of the specific dispersion isotherm, when the value of its ordinate is zero.

Young (85) found that the primary reaction caused by the irradiation of albumin solutions with visible light was accompanied by an increase in optical rotatory power. The rate of increase of optical rotatory power was increased by the addition of both acids and alkalies.

Spiegel-Adolf (66) found the dispersion coefficients for heated pseudo-globulin solutions to be 1.88 to 2.00, whereas the same coefficients for solutions irradiated with ultra-violet rays were 2.2 to 2.9. When the specific optical rotations were determined at different wave lengths and the results plotted on coördinate paper, the specific dispersion isotherms appeared to be almost linear. Adding alkalies changed the slope of the line, but the characteristic wave length remained the same. After irradiation, the characteristic wave length was markedly increased, as was the slope of the specific dispersion isotherm.

Zhukov and Unkovskaya (86) irradiated gelatin solutions with alpha-particles and found, in all cases, a decrease in the normal dextro-rotation of polarized light. The reaction of the solution did not qualitatively affect the finding. According to Ponthus (55) a similar result is obtained by irradiating gelatin with ultra-violet rays.

2. *Changes in Ultra-violet Absorption Spectra.* Stenström and Reinhard (77) have shown that the characteristic absorption band common to most proteins and having its peak at about 2800 Å. U. is due to the presence of one or more of the benzene rings containing amino acids. Spiegel-Adolf (67), Spiegel-Adolf and Krumpel (70, 71), Spiegel-Adolf and Oshima (69), and Hausmann and Spiegel-Adolf (33) found that exposure of proteins to radiant energy resulted in an increased absorption of ultra-violet light by the protein solutions. Becker and Szendiö (4) also found an increased absorption, this being accompanied, however, by a disappearance of the characteristic band at 2800 Å. U.

Arnow (1) has shown that the absorption of ultra-violet light by egg albumin solutions irradiated with alpha-particles is increased in the case of solutions at or below the iso-electric point, and is decreased in the case of solutions above the iso-electric point. The decreased absorption is interpreted as indicating a cleavage of the benzene ring, and it is suggested that the increased absorption might possibly be due to a conversion of tyrosine and phenylalanine to dihydroxyphenylalanine.

Becker (3) found that irradiation of l-proline with ultra-violet light resulted in the appearance of a new band at 3130 Å. U. The irradiation

of l-hydroxyproline caused the appearance of new bands at 2570 Å. U. and at 3130 Å. U.

3. *Changes in Viscosity.* Fernau and Pauli (26) and Zhukov and Unkovskaya (86) have reported that the viscosity of gelatin solutions decreases steadily under the influence of radium radiations. Ponthus (55) found that a similar decrease resulted when gelatin solutions were exposed to ultra-violet rays. Mond (50) reported the same to be true for globulin and albumin solutions exposed to ultra-violet radiation, but Young (85) observed that the viscosities of egg and serum albumin solutions were increased by sunlight and strong arc illumination. Wels (81) noticed an increase in the viscosities of globulin solutions and serum following exposure to Röntgen rays. Arnow's (1) experiments show that the viscosity of egg albumin solutions, exposed to alpha-particles, is increased if the protein solution is at or below the iso-electric point of egg albumin, but is decreased if the pH of the solution is above that of the iso-electric point.

4. *Changes in Coagulation Temperature.* The coagulation temperatures of globulins and fibrinogen are raised by exposure to ultra-violet rays, as Mond (50) and Arthus and Boshell (2) have reported. Mond found, however, that the coagulation temperatures of albumins were lowered by ultra-violet rays, and Fernau (24) obtained a similar result with the penetrating rays of radium. Stedman and Mendel (73) irradiated twelve purified proteins with ultra-violet rays and found that the coagulation temperatures of all the proteins studied were lowered. Arnow (1) exposed egg albumin solutions to alpha-particles and reported that the temperature of coagulation was lowered when the pH of the solution was equal to or greater than that of the iso-electric point, but was raised if the pH was lower than iso-electric.

5. *Changes in pH.* Young (85) and Mond (51) found that irradiation of albumins with visible or ultra-violet light caused the pH of the solutions to approach that of the iso-electric point, regardless of the initial pH. Clark (13), however, has maintained that irradiation of egg albumin with ultra-violet light always causes a decrease in the initial value of the pH. In an earlier paper, Mond (50) had found a decrease of pH after irradiation of solutions of globulin, fibrinogen, and albumin with ultra-violet light. Zhukov and Unkovskaya (86) found that the irradiation of gelatin solutions with alpha-particles resulted in a decrease in pH. Arnow's (1) work has shown that the pH of iso-electric solutions of egg albumin and of solutions of pH less than that of the iso-electric point is little affected by exposure to alpha-particles. If solutions of pH greater than iso-electric were irradiated, the pH was markedly lowered.

6. *Changes in the Gold Sol Reaction.* According to Mond (50), irradiation of globulin, fibrinogen, and albumin solutions with ultra-violet rays does not affect the protective action of globulin and fibrinogen solutions, but albumin solutions display increased protective powers. Spiegel-Adolf (67) irradiated weak solutions of serum albumin and pseudoglobulin with ultra-violet light. She offers evidence to show that proteoses and peptones are produced which have ten to forty times the protective ability of the original protein. When concentrated solutions of the proteoses obtained by the irradiation were added to normal spinal fluid, the mixture gave a gold sol reaction similar to that obtained in cases of general paralysis. This same worker later (68) reported that arginine, histidine, lysine, and proline flocculated gold sols after irradiation with ultra-violet light, although non-exposed amino acids did not do this. Gelatin, exposed to the rays, lost its protective power toward colloidal gold and precipitated the latter.

After adding gold sol to solutions of gelatin and egg albumin, Prideaux and Howitt (56) irradiated these solutions with ultra-violet rays. After sufficient irradiation, the proteins were flocculated around the gold micelles, and the cataphoretic velocities could then be measured in the ordinary way, the velocities measured appearing to be due to the proteins alone.

7. *Changes in Surface Tension.* The work of Mond (50), Young (85), and Wels (81) indicates that the irradiation of globulins and albumins with ultra-violet rays, visible light, and Röntgen rays has the effect of lowering the surface tensions of the protein solutions.

8. *Changes in Electrical Conductivity.* Ponthus (55) has found that exposure of gelatin solutions to ultra-violet rays causes an increase in the electrical conductivity of the solutions. Mascherpa (48) has reported that the conductivity of egg albumin solutions was increased by alpha-particle irradiation. In interpreting these results, however, it is well to remember that conductivity water itself undergoes an increase in electrical conductivity during exposure to alpha-particle irradiation, as the work of Nurnberger (53) has shown.

9. *Changes in the Refraction of Light.* Spiegel-Adolf (67) observed an increase in the refraction of light after exposure of pseudoglobulin and serum albumin solutions to ultra-violet rays. No such increase resulted if the proteins were denatured by heat.

C. CHEMICAL CHANGES. 1. *Oxidation-Reduction Reactions.* Oxyhemoglobin is converted to methemoglobin by alpha-particle irradiation, as shown by the experiments of Henri and Mayer (34) and of Chambers

and Russ (9). Fricke and Peterson (29) found that this same reaction occurred when oxyhemoglobin was irradiated with Röntgen rays, the rate of transformation being proportional to the momentary concentration of oxyhemoglobin. Bernard, Biancani, and Biancani (5) reported that carbon monoxide-hemoglobin disappeared during ultra-violet irradiation and offered evidence to show that their results could be explained by assuming the CO to be oxidized to CO_2. Lieben (42), Palit and Dhar (54), and Becker and Szendiö (4) discovered that amino acids could be oxidized by exposing them, in the presence of oxygen, to ultra-violet rays or to visible light. According to den Hoed, de Jongh, and Peek (15), insulin was oxidized by exposure to radium or ultra-violet rays, although exposure to Röntgen rays was without effect. Woodward's (84) work has shown that the SH group of glutathione can partially be oxidized to the SS form with both ultra-violet rays and with radium radiations, Röntgen rays having no such effect. Kinsey (37), however, found that Röntgen rays destroy glutathione. Neuberg (52) has reported that sunlight, in the presence of sensitizers, is capable of reducing amino acids to aldehydes, but later work by Spiegel-Adolf (68) does not support this. According to Arnow (1), the irradiation of egg albumin and gelatin solutions with alpha-particles resulted in a considerable utilization of oxygen by the protein molecule, the oxygen being supplied by the decomposition of water by the radiation. Hydrogen in smaller equivalent amounts was also utilized. The utilization of the hydrogen involved at least two reactions in the case of egg albumin and at least three reactions in the case of gelatin.

2. *Formation of Ammonia.* Effront (19) found that ammonia was produced when sterile peptone solutions were exposed to sunlight. Lieben and Urban (46) and Eckstein and Lieben (18) reported that ammonia appeared when amino acids or proteins were exposed to ultra-violet rays. Only the alpha-amino group was concerned. Stenström and Lohmann (74, 75, 76) detected the presence of ammonia in tyrosine and tryptophan solutions after irradiation with Röntgen rays, no ammonia resulting, however, from the irradiation of cystine for long periods of time. Loiseleur (47) has shown that the alpha-particle irradiation of amino acids results in ammonia formation.

3. *Removal of Halogen Groups.* Lieben and Ehrlich (43) reported that artificially halogenated casein, thyroxin, diiodotyrosine, and Merck's thyroidin all gave off iodide or bromide ions when treated with ultra-violet radiation.

4. *Destruction of the Benzene Ring.* Stenström and Lohmann (74,

75, 76) offered evidence to show that a destruction of the benzene rings of tyrosine and tryptophan occurred during Röntgen ray irradiation. Arnow (1) interpreted some of the changes in the absorption of ultra-violet light following exposure of egg albumin solutions to alpha-particles as indicating a splitting of the benzene ring.

5. *Miscellaneous Chemical Reactions.* Effront (19) demonstrated that hydrogen peroxide, nitrates, ammonia, and volatile acids were present in solutions of peptone which he had exposed to sunlight. Straub and Galwitzer-Meier (78), investigating carbon dioxide tension as a function of the carbon dioxide combining power of hemoglobin solutions, found certain irregularities which disappeared after alpha-particle irradiation. Holtz (35) reported that solutions of euglobulin or blood serum irradiated with ultra-violet light contained nitrous acid, acetic acid, and imidazole derivatives. Loiseleur (47) noticed that alpha-particles caused a liberation of urea from arginine, and a small amount of hydrogen sulphide was split from cystine by the radiation. Spiegel-Adolf (67, 68) produced proteoses and peptones by exposing weak solutions of blood proteins to ultra-violet rays. Lieben and Jesserer (44) irradiated Witte peptone with ultra-violet radiation and showed that the resulting insoluble product did not give the biuret reaction. Tryptophan disappeared and the amount of histidine was greatly diminished. Tyrosine was only slightly destroyed.

D. Physiological applications. 1. *The Production of Erythema.* Ellinger (20, 21, 22) found that irradiating solutions of histidine with ultra-violet light resulted in the formation of a substance which was capable of causing a fall in blood pressure. He believed this substance to be histimine, but Szendiö (79) has since given evidence to show that it is imidazoleacetaldehyde. Ellinger believes that this substance is formed during the production of an erythema by light and that this causes the first reddening.

According to Holtz (35), the exposure of serum, euglobulin, glycine, leucine, and tyrosine, as well as histidine, to the action of ultra-violet light results in the formation of substances which will cause dilatation of the blood vessels of the frog.

2. *Coagulation of the Lens Protein.* Burge (8) found that ultra-violet light did not coagulate the proteins of aqueous humor, the vitreous body, or of the lens. If, however, calcium chloride, magnesium chloride, sodium silicate, or glucose were added to the medium in which the lens was irradiated, opacity resulted. Since some cataracts have increased amounts of calcium, magnesium, and silicate, Burge assumed that these

types of cataract were most probably caused by radiant energy. He believed the lenticular opacity which often accompanies diabetes mellitus to be the result of the action of radiation on a lens which contained an abnormal amount of glucose. Schanz (60) thought that acetone, acting as a sensitizer, might be responsible for the lenticular opacity of diabetes. However, Lieben and Kronfeld (45) were unable to demonstrate a loss of tryptophan from the protein of the lens after exposure to sunlight, and came to the conclusion that the ultra-violet light probably was filtered out by the outer layers of the lens. Lieben (41, 42) had previously shown that tryptophan and tyrosine were destroyed when proteins in pure solution were irradiated.

3. *Biological Oxidations and Reductions.* Wels and his associates (40, 82, 83) have shown that the irradiation of egg albumin, in an oxygen free atmosphere, with ultra-violet radiation results in the production of a substance which contains free SH groups and which is reversibly oxidizable. This substance apparently is attached to the protein molecule. In an oxygen stream the ability of the irradiated protein to bleach methylene blue is lost, but this ability is regained by allowing the oxygenated product to remain in contact with washed frog muscle, or dry powdered muscle, for a few hours. As already mentioned, Woodward (84) found that ultra-violet light and radium rays can oxidize SH groups to the SS form. Wels believes it probable that the irradiation of skin results in the formation of a substance possessing the SH group and playing a rôle in tissue oxidations and reductions.

4. *Miscellaneous Physiological Effects.* As might be expected, both Ellinger and Holtz (see above) have noticed that the substances produced in their experiments will stimulate the intestine.

Holtz (36) has found that irradiated serum also markedly affects the elimination of sugar by the liver of guinea pigs, cats, and dogs. In the majority of his experiments, the elimination was reduced.

REFERENCES

(1) Arnow. J. Biol. Chem. **110**: 43, 1935.
(2) Arthus and Boshell. Arch. Intern. Physiol. **30**: 244, 1928.
(3) Becker. Strahlentherapie **48**: 296, 1933.
(4) Becker and Szendiö. Pflüger's Arch. **228**: 755, 1931.
(5) Bernard, Biancani and Biancani. Compt. rend. Soc. biol. **92**: 1031, 1925.
(6) Bovie. Science **37**: 24, 1913.
(7) Bovie. Science **37**: 373, 1913.
(8) Burge. Am. J. Physiol. **36**: 21, 1915.
(9) Chambers and Russ. Proc. Roy. Soc. London **84B**: 124, 1912.
(10) Clark. Physiol. Rev. **2**: 277, 1922.

(11) CLARK. Am. J. Hyg. **2**: 322, 1922.
(12) CLARK. Am. J. Physiol. **61**: 72, 1922.
(13) CLARK. Am. J. Physiol. **73**: 647, 1925.
(14) CLARK. J. Gen. Physiol. **19**: 199, 1935.
(15) DEN HOED, DE JONGH AND PEEK. Biochem. Ztschr. **205**: 144, 1929.
(16) DREYER. Trans. Oxford Univ. Jun. Sci. Club (*3*) no. 7: 212, 1922.
(17) DREYER AND HANSSEN. Compt. Rend. Acad. Sci. **145**: 234, 1907.
(18) ECKSTEIN AND LIEBEN. Biochem. Ztschr. **263**: 366, 1933
(19) EFFRONT. Compt. rend. **154**: 1111, 1913.
(20) ELLINGER. Arch. exper. Path. u. Pharmakol. **136**: 129, 1928.
(21) ELLINGER. Arch. exper. Path. u. Pharmakol. **153**: 120, 1930.
(22) ELLINGER. Strahlentherapie **38**: 521, 1930.
(23) FERNAU. Kolloid Ztschr. **33**: 89, 1923.
(24) FERNAU. Biochem. Ztschr. **167**: 380, 1926.
(25) FERNAU AND PAULI. Biochem. Ztschr. **70**: 426, 1915.
(26) FERNAU AND PAULI. Kolloid Ztschr. **30**: 6, 1922.
(27) FERNAU AND SPIEGEL-ADOLF. Klin. Wchnschr. **6**: 1798, 1927.
(28) FERNAU AND SPIEGEL-ADOLF. Biochem. Ztschr. **204**: 14, 1929.
(29) FRICKE AND PETERSON. Am. J. Roentgenol. **17**(6): 611, 1927.
(30) GALINSKY. Biochem. J. **24**: 1706, 1930.
(31) GENTNER AND SCHWERIN. Biochem. Ztschr. **228**: 286, 1930.
(32) HARDY. Proc. Physiol. Soc. (**29**, J. Physiol.) xxiv, 1903.
(33) HAUSMANN AND SPIEGEL-ADOLF. Klin. Wchnschr. **6**: 2182, 1927.
(34) HENRI AND MAYER. Compt. rend. Soc. biol. **55**: 1412, 1903.
(35) HOLTZ. Pflüger's Arch. **226**: 559, 1931.
(36) HOLTZ. Pflüger's Arch. **226**: 578, 1931.
(37) KINSEY. J. Biol. Chem. **110**: 551, 1935.
(38) KRUYT AND BUNGENBERG DE JONGH. Ztschr. physik. Chem. **100**: 250, 1922.
(39) KRUYT AND BUNGENBERG DE JONGH. Koll. Beih. **28**: 1, 1928.
(40) LAMPERT AND WELS. Arch. exper. Path. u. Pharmakol. **175**: 554, 1934.
(41) LIEBEN. Biochem. Ztschr. **184**: 453, 1927.
(42) LIEBEN. Biochem. Ztschr. **187**: 307, 1927.
(43) LIEBEN AND EHRLICH. Biochem. Ztschr. **222**: 221, 1930.
(44) LIEBEN AND JESSERER. Biochem. Ztschr. **275**: 367, 1935.
(45) LIEBEN AND KRONFELD. Biochem. Ztschr. **197**: 136, 1928.
(46) LIEBEN AND URBAN. Biochem. Ztschr. **239**: 250, 1931.
(47) LOISELEUR. Compt. rend. Soc. biol. **114**: 589, 1933.
(48) MASCHERPA. Boll. Soc. ital. biol. sper. **8**: 617, 1933.
(49) MEISLING. Bull. Acad. roy. Denemark 167, 1908.
(50) MOND. Pflüger's Arch. **196**: 540, 1922.
(51) MOND. Pflüger's Arch. **200**: 374, 1923.
(52) NEUBERG. Biochem. Ztschr. **13**: 305, 1908.
(53) NURNBERGER. Thesis, Univ. of Minnesota, 1932.
(54) PALIT AND DHAR. J. Physiol. Chem. **32**: 1263, 1928.
(55) PONTHUS. Compt. rend. **196**: 1248, 1933.
(56) PRIDEAUX AND HOWITT. Biochem. J. **25**: 391, 1931.
(57) RAJEWSKY. Strahlentherapie **33**: 362, 1929.

(58) RAJEWSKY. Biochem. Ztschr. **227**: 272, 1930.
(59) RAYBAND. Compt. rend. Soc. biol. **68**: 381, 1910.
(60) SCHANZ. Pflüger's Arch. **164**: 445, 1916.
(61) SCHANZ. Pflüger's Arch. **170**: 646, 1918.
(62) SPIEGEL-ADOLF. Biochem. Ztschr. **186**: 181, 1927.
(63) SPIEGEL-ADOLF. Strahlentherapie **29**: 367, 1928.
(64) SPIEGEL-ADOLF. Biochem. Ztschr. **197**: 197, 1928.
(65) SPIEGEL-ADOLF. Klin. Wchnschr. **7**: 1592, 1928.
(66) SPIEGEL-ADOLF. Biochem. Ztschr. **213**: 475, 1929.
(67) SPIEGEL-ADOLF. Biochem. J. **28**: 372, 1934.
(68) SPIEGEL-ADOLF. Biochem. J. **28**: 1201, 1934.
(69) SPIEGEL-ADOLF AND OSHIMA. Biochem. Ztschr. **208**: 32, 1929.
(70) SPIEGEL-ADOLF AND KRUMPEL. Biochem. Ztschr. **190**: 28, 1927.
(71) SPIEGEL-ADOLF AND KRUMPEL. Biochem. Ztschr. **208**: 45, 1929.
(72) SPIEGEL-ADOLF AND POLLACZEK. Biochem. Ztschr. **214**: 175, 1929.
(73) STEDMAN AND MENDEL. Am. J. Physiol. **77**: 199, 1926.
(74) STENSTRÖM AND LOHMANN. J. Biol. Chem. **79**: 673, 1928.
(75) STENSTRÖM AND LOHMANN. Radiology **13**: 437, 1929.
(76) STENSTRÖM AND LOHMANN. Radiology **17**: 432, 1931.
(77) STENSTRÖM AND REINHARD. J. Biol. Chem. **66**: 819, 1925.
(78) STRAUB AND GALLWITZER-MEIER. Biochem. Ztschr. **136**: 128, 1923.
(79) SZENDIÖ. Pflüger's Arch. **228**: 743, 1931.
(80) TIAN. Compt. rend. **151**: 219, 1910.
(81) WELS. Pflüger's Arch. **199**: 226, 1923.
(82) WELS. Arch. exper. Path. u. Pharmakol. **171**: 480, 1933.
(83) WELS AND HESSE. Naturwissenschaften **22**: 648, 1934.
(84) WOODWARD. Biochem. J. **27**: 1411, 1933.
(85) YOUNG. Proc. Royal Soc. London **93B**: 235, 1922.
(86) ZHUKOV AND UNKOVSKAYA. J. Russ. Physiol. Chem. Soc. **62**: 581, 1930.

PHYSIOLOGICAL REVIEWS

Contents of Volume 14, 1934

E. K. Marshall: The Aglomerular Kidney
L. B. Flexner: The Chemistry and Nature of the Cerebrospinal Fluid
H. Jensen and E. A. Evans, Jr.: The Chemistry of Insulin
R. G. Sinclair: Physiology of the Phospholipids
S. W. Clausen: Diet and Infection
W. S. Duke-Elder: Application of Physiological Chemistry in the Study of Intraocular Pressure
J. M. Peterson: Metabolism of the Eviscerate Preparation
E. M. Landis: Capillary Pressure and Capillary Permeability
J. C. Hinsey: The Innervation of Skeletal Muscle
G. A. Alles: The Physiological Significance of Choline Derivatives
R. C. Garry: The Movements of the Large Intestine
A. C. Ivy: Physiology of the Gall Bladder
S. Mudd, M. McCutcheon and B. Lucke: Phagocytosis

Contents of Volume 15, 1935

O. R. McCoy: Physiology of Animal Parasites
C. L. A. Schmidt and D. M. Greenberg: Transport of Alkaline Earth Elements in the Animal Body
C. M. Wilhelmj: Specific Dynamic Action
W. F. von Oettingen: Manganese, Its Distribution, Pharmacology and Health Hazards
W. H. Howell: Present Status of the Problem of Blood Coagulation
W. E. Garrey and W. R. Bryan: Variations in White Blood Cell Counts
C. A. Elvehjem: Iron and Copper Metabolism
C. E. Bills: Physiology of Sterols and the Influence of Light on Sterols
W. M. Hoskins and R. Craig: Recent Developments in Insect Physiology
M. N. Richter and E. C. McDowell: Experiments with Mammalian Leukemia
P. Weiss: Organizers in Embryological Development
F. J. W. Roughton: Recent Work on CO_2 Transport
R. J. S. McDowall: The Nervous Control of the Blood Vessels

Contents of Volume 16, 1936

J. Barcroft: Fetal Circulation and Respiration
W. J. V. Osterhout: Electrical Studies on Large Plant Cells
W. O. Fenn: Electrolytes in Muscle
A. N. Drury: Physiological Activity of Nucleic Acid Derivatives
R. Höber: Membrane Permeability to Solutes in Its Relations to Physiology
L. B. Arey: Mechanism of Wound Healing
H. Davis and A. Forbes: Chronaxie
A. P. Krueger: The Nature of Bacteriophage and Its Mode of Action
C. G. King: Ascorbutic Acid and Vitamin C
H. J. Deuel: Metabolism of Fructose and Galactose
W. O. Nelson: Lactation
D. B. Dill: Economy of Muscular Activity
R. S. Alcock: The Synthesis of Proteins in Vivo
John H. Ferguson. The Blood Calcium and the Calcium Factor in Blood Coagulation
H. G. Wolff: The Cerebral Circulation
E. W. H. Cruickshank: Metabolism of Cardiac Muscle
H. R. Ing: Curariform Action of Onium Salts
L. Earle Arnow: Effects Produced by the Irradiation of Proteins and Amino Acids.
G. Bourne: The Rôle of Vitamin C in the Organism as Suggested by Its Cytology
E. Ponder: The Kinetics of Hemolysis

PHYSIOLOGICAL REVIEWS

Tentative Contents of Volume 17, 1937

S. R. M. Reynolds: Control of Uterine Motility

E. E. Nelson and H. O. Calvery: The Present Status of the Ergot Problem

Raphael Isaacs: Formation and Destruction of Red Blood Cells

Leslie Hellerman: The Reversible Inactivation of Hydrolytic Enzymes

C. B. Huggins: Physiology of Bone

Selig Hecht: Chemistry of Vision and Photoreception

H. K. Hartline: Electrical Studies of Visual Mechanisms

Sarah S. Tower: Degeneration in Skeletal Muscle

F. R. Winton: Physical Factors Involved in the Activites of the Mammalian Kidney

Leo Loeb: Physiological Old Age

V. E. Henderson and M. H. Roepke: Drugs Affecting Parasympathetic Nerves

W. G. MacCallum: Physiological Pathology of the Prostate

A. E. Severinghaus: Cellular Changes in the Anterior Hypophysis With Special Reference to its Secretory Activities

M. C. Bourne: Metabolic Factors in the Production of Cataract

J. R. Murlin: Some New Factors of Energy Metabolism

G. W. Bartelmez: Menstruation

D. W. Bronk: Unitary Analysis of Reflex Activity

P. Bard: Functions of the Hypothalamus

H. S. Liddell: Recent Contributions to the Physiology of the Conditioned Reflex

W. H. Chambers: Undernutrition and Carbohydrate Metabolism

J. F. Fulton and M. A. Kennard: Representation of Autonomic Functions in the Cerebral Hemispheres

J. H. Northrop: The Formation of Enzymes

William Bloom: Cell Differentiation in Tissue Cultures

G. L. Brown and W. Feldberg: Transmission at Nerve Endings by Acetylcholine

A. Rosenblueth: The Transmission of Sympathetic Nerve Impulses

J. C. Eccles: Synaptic and Neuromuscular Transmission

A. G. Bills: Fatigue in Mental Work

H. H. Woollard: Specificity in Structure and Function of Nerve Endings of the Skin

Helen Tredway Graham: The Significance of the Potentials Manifested During Nervous Activity

Hugo Krueger: The Action of Morphine on the Diegestive Tract

W. C. Rose: Nutritive Significance of Amino Acids

Phyllis Tookey Kerridge: Physiology of Hearing and Speech

A. Baird Hastings: Electrolyte Equilibria in the Blood and Body Fluids

F. Gowland Hopkins: Glutathione

William E. Anderson and H. H. Williams: Rôle of Fat in the Diet

C. P. Richter: The Psycho-Galvanic Reflex

E. S. G. Barron: Cellular Respiration

R. K. Burns: Experimental Transformations of Sex in Vertebrates

J. J. Morton and W. J. M. Scott: Sympathetic Vasoconstriction in Normal and Pathological Arteries of the Extremities

F. S. Hisaw and H. L. Fevold: Sex Hormones of Anterior Pituitary

Carl L. Voegtlin: Biochemistry of Malignant Tissues

F. C. Koch: The Male Sex Hormone